

**THE POTENTIAL OF WATER FROM KIPLOLOK BUBBLING SPRINGS IN
NANDI COUNTY FOR USE AS NATURAL CARBONATED MINERAL WATER**

BY

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DECLARATION

DECLARATION BY THE CANDIDATE

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ABSTRACT

The demand for carbonated water in Kenya, just like the rest of the world, is on the rise, owing to increasing lifestyle diseases like obesity, which requires that for a healthy lifestyle a lot of carbonated water, should be taken throughout the day. This research was set to determine the quality of water from Kiplolok bubbling springs in Nandi County, Kenya. In addition, the research was to determine whether the water can be used as natural carbonated water. Six sampling points were identified; three bubbling points labeled as (P1, P2, and P4), a shallow well (P3), river (P5), and a spring (P6). A total of 60 samples were collected during the dry and rainy seasons. The samples were used for determination of selected cations and anions, indicator bacteria, carbonation and physico-chemical properties. The results indicated the water was contaminated with faecal and total coliforms in all the sampling points P1, P2, P3, P4, P5, and P6. High turbidity was recorded in sampling points P1, P2, P4, while high amounts of iron and manganese above those recommended by WHO and KEBS for drinking water were recorded in most sampling points. The water recorded low pH values in both seasons for all but P5 sampling points with 6.8 and 6.37 in the rainy and dry seasons. The pH values for the other samples ranged between 3.41 -4.93 in the rainy season and 3.8-5.25 in the dry season. KEBS pH standards for carbonated water are at minimum pH of 4. The dissolved CO₂ concentration of the water ranged from 1.20 g/L to 2.65 g/L in the rainy season and 1.50 g/L to 3.00 g/L in the dry season for all sampling points, conforming to the KEBS standards for carbonated water of at least 1 g/L but P5 recorded CO₂ values of 0.5 and 0.4 g/L for rainy and dry season respectively. In conclusion, the results showed the water from all the sampling points except P5, contained sufficient dissolved carbon dioxide for use as naturally carbonated water. However, purification and treatment is necessary to lower the turbidity of the water, eliminate the microbial contaminants and adjust pH to at least 4, to conform to the KEBS recommendation for naturally carbonated water.

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LIST OF ABBREVIATIONS

- EPA - Environmental Protection Agency
- FDA - Food and Drug Administration
- GERD - Gastro-Oesophageal Reflux Disease
- GHHIM- Global Hidden Hunger Indices and Maps
- HHIS - Hidden Hunger Index Score
- KBS - Kiplolok Bubbling Springs
- KEBS - Kenya Bureau of Standards
- MCLs – Maximum Concentration Levels
- NMW - Natural Mineral Water
- pH – Potential Hydrogen
- PPM – Parts Per Million
- TDS - Total Dissolved Solids
- UNWDR- United Nations Water Development Report
- WHO - World Health Organisation

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CHAPTER ONE

INTRODUCTION

1.1 Background

Water is used in ensuring hygiene – it is used in keeping waterborne diseases such as viral hepatitis and dysentery at bay. Water assists the human body in excreting waste, especially in sweat and urine (Rosborg, 2014). Lymph nodes have substantial amounts of water, which helps buildup and sustain one's immunity (Moore & Bertram, 2018). However, carbonated mineral water is known to have essential minerals of health benefit to the body, for example, Naumann, Sadaghiani, Alt & Huber,(2016) assert that sulfate-rich mineral water osmotically hinders water absorption in the large bowel and accelerates intestinal transit, ensuring better stool consistency. Sulfate-rich mineral water is thus very useful in easing constipation-related complications.

Water for human use can either be treated or not treated and is intended for drinking, beverage and food preparation, and other domestic uses. Natural mineral waters are among the waters intended for these purposes. These waters originate from underground reservoirs or aquifers; they may as well emanate from springs with one or more natural bore sources. Natural mineral water sources must have specific hygienic features and well-known health benefits according to the 2009/54/EC Directive of the European Commission; classification of natural mineral water is done based on the chemical characteristics and physical properties of the water; as such, laboratory analysis must be done before the classification of these waters (Sara, Barbara & Maria, 2016).

The most known categories of natural mineral water include bicarbonate mineral water, chloride mineral water, magnesia mineral water, ferrous mineral water, sulfate mineral water, fluorurate mineral water, sodium-rich mineral water as well as calcic mineral water. Natural mineral water has also been classified based on the presence or absence of carbon dioxide (CO₂) and the mode of CO₂ introduction. The carbonation of sparkling water also referred as carbonated water can occur naturally or artificially. Natural carbonated water or sparkling water is formed naturally when volcanic gases dissolve in springs or wells water, thus carbonating the water. However, when carbon dioxide is artificially added, the water is commonly referred to as carbonated water instead of natural carbonated water.

In Italy, natural mineral water is differentiated from drinking water based on the purity and conservation of the spring as well as constant mineral levels, both trace, and other constituents, and from the health and other benefit conferred by such water (Italy, D. Leg. N. 176 of 2011). The Kenya Bureau of Standards (KEBS) has adopted the same criteria here in Kenya. The Food and Drug Administration (FDA) in the United States classifies bottled water and regulates it as food, and also it identifies natural mineral water as “water containing not less than 250 parts per million (ppm) total dissolved solids (TDS), coming from a source at one or more bore holes or springs, originating from a geologically and physically protected underground water source” (Code of Federal Regulations, Title 21, Part 165 - Beverages) and (FDA, 2019)

Notably, in its state at source, natural mineral water must not be the subject of any treatment or addition other than the addition or elimination of carbon dioxide. Natural

mineral water are rich in minerals such as magnesium, calcium, potassium, iron, and sodium. Unlike regular drinking water, natural mineral water is often bottled at the source (Gałarska, Ciborska & Tońska, 2016).

Regularly drinking natural mineral water daily contributes significantly to the general well-being of individuals. This is because the human body uses minerals from natural mineral water to perform various integral functions (Petraccia *et al*, 2006).

Some natural mineral water are rich sources of magnesium (Gałarska, Ciborska & Tońska, 2016). Magnesium is an extremely vital mineral that helps in regulating blood pressure, blood glucose levels, and nerve function in the human body. As such, regularly drinking natural mineral water rich in magnesium alternatively aids in lowering the risk of cardiovascular diseases.

Moreover, the intake of magnesium by drinking natural mineral water essentially contributes to good health in other ways in the body; first, magnesium possesses anti-inflammatory benefits, which is integral in fighting against chronic diseases in the body. Secondly, research has demonstrated the essence of magnesium in fighting type 2 diabetes mellitus disease. This is because supplementation of magnesium through drinking natural mineral water aids in improving insulin sensitivity and metabolic control against type 2 diabetes mellitus in the body (Gałarska, Ciborska & Tońska, 2016).

Natural mineral water may contain large amount of magnesium, calcium, and potassium, all of which aid in promoting blood circulation in the body (Gałarska, Ciborska & Tońska, 2016). These minerals are vital in regulating the rate and rhythm of the heartbeat.

Carbonated mineral water significantly aids in relieving symptoms of constipation and indigestion. As an added benefit, one study demonstrated how carbonated mineral water exhibited the highest ability to stimulate swallowing nerves, thus positively impacting digestion (des Gachons *et al.*, 2016).

A 2004 study revealed that drinking plenty of carbonated mineral water aids in significantly lowering blood pressure in individuals (Rylander & Arnaud, 2004).

Natural carbonated mineral water is a healthier alternative to fizzy soft and sports drinks. Unlike fizzy soft and sports drinks, Natural carbonated mineral water does not have added sugars, which can cause weight gain and tooth decay (des Gachons *et al.*, 2016). Reports indicate that drinking carbonated mineral water often provides the same sensation as drinking a fizzy soft and sports drink without the added calories and sugar.

Water in springs is expected to be safe for human consumption. However, it may contain gases, dissolved minerals, chemicals, and microbial organisms that make it unsafe for human consumption. Kenya is one of the developing countries where people face an acute shortage of safe drinking water (Mulwa, Li & Fangninou, 2021). According to Water.org 2020 report 16 million Kenyans lack access to clean and safe drinking water. A significant number of people, mainly in rural areas, are dependent on unprotected water sources such as springs, rivers, and hand-dug wells for their domestic consumption (Mulwa, Li & Fangninou, 2021). In light of this, it was vital to undertake an analysis of water from Kiplolok, Kilibwoni bubbling springs in Nandi County, Kenya. Indeed according to the local community, this water tastes like sprite soda or tonic water,

alluding to the fact that the bubbling of the water may be due to the high pressure of dissolved gases emanating from geological activities and processes underground.

1.2 Statement of the Problem

Mineral deficiency-related diseases and lifestyle diseases pose a serious health challenge. According to the Kenya strategy on non-communicable diseases, NCDs under which most of the lifestyle diseases such as high blood pressure, heart diseases and diabetes fall, causes of 63% of the deaths globally, 80% of which occur in developing countries. On the other hand there has been an increase in the cases of anemia especially maternal anemia. Odhiambo and Sartorius (2020) reported that the number maternal anemia increased by 90.1% between 2016 -2019, for women visiting sub county hospitals in Kenya.

As the nutritionists call it, hidden hunger is a serious problem in Kenya. For example, the Global Hidden Hunger Indices and Maps (GHHIM) of 2014 ranked Kenya second out of the 20 countries considered, with a hidden hunger index score (HHIS) of 51.7% (International Food Policy Research Institute, 2014). Although vegetables and fruits are the main sources of these micronutrients essential for growth and healthy well-being, the escalating prices of these commodities have been a hindrance to the fight against hidden hunger. Commercial food supplements are expensive and not available to most Kenyans, especially in rural areas. With proper cleaning and purification, Kiplolok water will serve as an affordable source of carbonated mineral water.

1.3 Justification for the study

Over the years numerous studies have been carried out to determine quality of water in diverse water sources; including rivers, borehole, wells, and lakes as well as in springs. Heavy metal pollution, pesticides and other anthropogenic pollution of water have been studied extensively (Mwaniki et al., 2013). However, there are very few studies that have been carried out to determine potential uses and economic importance of some of the spring and underground waters that have special characteristics. Waters from Kiplolok bubbling water are special, unlike the hot geyser water that bubble owing to their high temperature and heat, Kiplolok water are cold and the bubbling is suspected to be due to gases emanating from underneath. On the other hand the water has a unique taste of “soda water”. It was therefore important to ascertain the quality of the water, and determine its potential for use as a source of natural carbonated mineral water. Given that the cost associated with water mineralization is relatively high (Donovan, 2014)

1.4 Objectives of the study

The broad objective of the study was to analyze varied water samples collected from Kiplolok Bubbling Springs (KBS), and establish whether this water can be used as carbonated Mineral Water (NMW), by comparing the results with those set by 2009/54/EC Directive for natural mineral water and natural carbonated water. The study sort to realize various specific objectives:

- i. Determine the concentration of selected cations and anions in Kiplolok Bubbling Springs water.
- ii. Establish the levels of fecal and total coliform bacteria

- iii. Determine whether Kiplolok Bubbling Springs water is naturally carbonated by analyzing the carbon dioxide and pH levels
- iv. Determine the levels physico-chemical parameters of Kiplolok Bubbling Springs water such as temperature, electrical conductivity, and turbidity.
- v. Establish variations between sampling seasons and sampling sites using single factor ANOVA

1.5 Research questions

- i. What is the concentration of selected cations and anions in KBS water?
- ii. What are the fecal and total coliforms concentrations in Kiplolok Bubbling Springs water?
- iii. What are the pH and carbon dioxide concentrations in Kiplolok Bubbling Springs water?
- iv. What are the levels physico-chemical parameters of Kiplolok Bubbling Springs water?
- v. How do the results obtained vary between seasons and between sampling sites

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

This chapter reviews the extant bodies of literature related to varied aspects of natural mineral water. Some of the aspects are definition and classification of natural mineral water, permitted physico-chemical, chemical and microbial parameters of water, benefits of elemental minerals found in natural mineral water, theoretical aspect of microbial and chemical analytical methods to be used in the analysis of this water, chemistry, and uses of water. It also seeks to establish whether there are past scientific studies related to the subject matter of the proposed study. By and large, this chapter presents results on extensive research into natural carbonated mineral water.

2.2 Definition of natural mineral water

Definition of natural mineral water can be traced back to 1980 when the European Economic Community first published a directive concerning exploitation as well as the marketing of natural mineral waters (Petraccia *et al.*, 2006). This comprehensive and authoritative document is the basis of most definitions of terms and phrases used to date. Natural mineral water is defined as microbiologically wholesome water originating from an underground water table or aquifer emerging from a spring with one or more natural or bore exits. However, it is imperative to note that the term microbiologically wholesome should not be taken literally but guarantee the absence or elimination of the main contamination indicators, including sporulated sulfite reducing anaerobes, faecal streptococci, *Pseudomonas aeruginosa*, *Escherichia coli*, as well as pathogenic and parasitic microorganisms (Astel, 2016). On the other hand, KEBS defines natural potable

water or natural mineral water as “water from natural sources that is fit for human consumption without undergoing any form of treatment, which will alter its original chemical composition or bacteriological purity” Water from a natural source that contains few mineral or low mineral concentrations is referred to as spring water.

Natural mineral water is classified based on its mineral composition. These classifications include magnesiatic, bicarbonate, sulfate, chloride, nitrate, manganese, ferrous, fluorurate, calcic and/or sodium-rich mineral waters (Sara, Barbara & Maria, 2016). The classification of mineral waters is vital in determining the health benefits of each type of mineral water. For instance, ferrous mineral water can be used to treat anaemia. On the other hand, magnesiatic mineral water with higher magnesium concentration help Oddi sphincter relaxation and facilitate bile flow, which improves the activity of biliary ducts. However, some natural mineral waters may also have toxic effects. For instance, fluorurate mineral waters may cause dental and skeletal fluorosis if the level of fluoride intake is more than 10 mg/L (Sara, Barbara & Maria, 2016). Water can as well be classified as naturally carbonated if the water is naturally carbonated from the source; carbon dioxide is naturally added through geological and volcanic processes underground, carbonated water where carbon dioxide is added from a different origin, and decarbonated water where it contains less than carbon dioxide that was present at the source. The water can as well be classified as non-carbonated, this is water that contains zero or free of carbon dioxide (Codex Standard, 2013 p. 108)

2.3 Classification of natural mineral water

2.3.1 Calcic Mineral Waters

In calcic mineral water calcium is the principal mineral. Heaney (2006), notes that adequate calcium intake is crucial for skeletal health; besides, this mineral may also be useful for other nonskeletal body systems, including the muscle, blood and nervous system. According to the WHO, inadequate intakes of calcium can lead or is associated with an increase in several ailments, most of which can be managed but lack an absolute cure. These ailments include osteoporosis, colorectal cancer, nephrolithiasis, stroke, hypertension, insulin resistance, coronary heart disease (Chao *et al.*, 2019). However, there lacks sufficient data and, consequently, compelling evidence on the role of calcium in most of the diseases mentioned above. For this reason, daily calcium intake requirements and general requirements for calcium are based on healthy bone outcomes to optimize bone mineral density.

Calcium is rarely found in the extracellular liquid - only about 0.1% - 1% of the total calcium is found in human body cells, with the rest being stored in the teeth and bones. Indeed the bones and teeth store 99% of calcium which works as a core structural element. Increasing bone mass has a linear relationship with the reduction in fracture risks. The total body calcium reservoirs are in the order of 1200g, with over 98% of this being found in bones and teeth (Chao *et al.*, 2019). Increased intake of calcium, especially among those who had habitual low calcium intake, greatly increases the bone mass during growth and consequently reduces fracture risks as well as bone loss late in life. Calcium content in natural carbonated mineral water is vital in bone development (Chao *et al.*, 2019). Calcium is often integral in human bodies because it possesses bone-

boosting benefits (Gałarska, Ciborska & Tońska, 2016). Ferreira (2016) note that the absorption of calcium from mineral waters by the human body is similar to that of calcium found in the milk, and thus taking calcic natural mineral water can be an effective and supplementary alternative to taking milk or even better due to its comparable if not better bioavailability. Bacciottini *et al*, 2004 reported that the absorption percentage of 3.18mmol of calcium for water was 22.53 ± 2.53 for men and 21.62 ± 3.12 for postmenopausal women and 22.57 ± 2.10 for premenopausal women, while that for milk was reported at 23.15 ± 4.06 (mean \pm SD)

2.3.2 Magnesiatic Mineral Water

Magnesium is a common ion that is present in any living cell. Magnesium plasma concentration levels are relatively stable in a healthy subject. Indeed magnesium plays vital role in the structure as well as in the function of the human body. About 25 grams of magnesium is present in an adult human body. Of all the body magnesium over 60% is found in the skeleton, 27% in the muscles and about 7% of this in the cells, leaving only a mere 1% present outside the cells. Magnesium is among the leading elements in the human body; it is the fourth cation in abundance and the second intracellular fluid (National Institutes of Health, 2020). Its importance can't be underestimated, it is a cofactor to over 300 cellular enzymes, a majority of which are associated with energy metabolism, for instance, ATP and glycolysis.

On the other hand, it varies signal transduction and cell growth. This metal ion also acts as a cofactor in nutrients and ions, including calcium, potassium, and sodium, across the membranes; it is involved in nucleic acid and protein synthesis. It is as well crucial if

normal insulin sensitivity and apt vascular tone are to be maintained as it has been proven to take part in neuromuscular excitability and muscle contraction (National Institutes of Health, 2020). Magnesium is integral in preventing constipation and improving the health of the digestive system (Dupont, Campagne & Constant, 2014). According to a study report by Bothe Coh and Auinger (2017), drinking natural mineral water rich in magnesium was established to promote more frequent bowel movement, which ultimately improved stool consistency for the respondents.

Magnesium, unlike most minerals, is very common, given that it is available in most foods; vegetables, fruits, legumes as well as in other animal products. However, acute deficiency of this ion may present with nausea, vomiting, anorexia, apathy, and general body weakness. A severe deficiency will lead to muscle cramps, irritability, loss of attention, and mental confusion. The total reserve for magnesium is about 25g, and the bone tissues are the main reservoir for this mineral. In humans, absorption takes place in the jejunum and the ileum. The intestinal bioavailability of this mineral depends on, among other factors, the deposits of the magnesium in the body, the amount of active ingredient, the type of dose, as well as the type of the salt (Abdullah, Sandawana & Henrik, 2018). The dieticians and nutritionists allude that mineral water rich in magnesium can go a long way in helping achieve the recommended daily intake of this mineral.

2.3.3 Ferrous mineral water

Ferrous mineral water is rich in iron which is important for body tissues and organs, as well as brain function. It also plays a vital role in regulating body fluids and hormones.

Iron is a fundamental mineral required for growth and development. By making use of iron, the body makes both hemoglobin and myoglobin proteins that are essential for supply oxygen in the cells and muscles respectively. Study reports have revealed how drinking water rich in natural minerals such as iron has been used to prevent iron deficiency in children (Petraccia *et al.*, 2006).

Other beneficial outcomes of drinking iron-rich mineral water include boosting and strengthening the immune system activity, regulating body temperature, prevention or treatment of anemia, boosting the gastrointestinal processes, enhancing energy levels in the body, and improving cognitive development and growth in young children (Petraccia *et al.*, 2006).

2.4 Cations and Anions

2.4.1 Sodium (Na⁺)

Sodium is among the most profuse elements on earth, coming at position number six; it is widely distributed in plants, soil, and water. Thus, the earth has large deposits of sodium-containing minerals, and, as such, this element is ever-present in water due to the high solubility of the majority of sodium salts (EPA, 2004). Sodium is always present in the ground and spring waters. This element is an important constituent at relatively low concentration. Sodium is an essential element involved in the continuance of normal cellular homeostasis, electrolyte and fluid balance (Strazzullo & Leclercq, 2014), and blood pressure regulation. Besides, sodium is important in the proper functioning of muscles and nerve impulses in the body. However, at concentrations above 200mg/L, sodium results in the salty taste of water and are unsuitable to persons with congestive

heart failure, immature kidneys as well as to those with hypertension due to salt retention. Waters with concentrations of 400mg/L and above are deemed unsuitable, more so for the infants (Ashun, 2017). KEBS (2015) and WHO, (2017) recommend that the sodium concentration in drinking water need not exceed 200mg/L.

2.4.2 Chloride (Cl⁻)

Naturally, chlorides occur as salts of Calcium (CaCl₂) sodium (NaCl) or potassium (KCl). Chloride is important in assessing and determining water quality; high concentration of this anion signifies a higher level of organic pollution. KEBS (2015) gives a guideline value of 250mg/L of chloride as the permitted limit in drinking water. Concentrations higher than the recommended ones; have been known to cause diarrhea and dehydration, gastrointestinal problems, and sometimes irritation (Ontumbi, Obando & Ondieki 2015).

2.4.3 Nitrates (NO₃⁻)

Being part of the nitrogen cycle, nitrate is a naturally occurring anion. It is as a result of nitrogen oxidation in the environment. This anion is highly stable as compared with nitrite; it is chemically unreactive, although it can be reduced by microbial action. According to the WHO, nitrate is used mainly in the agricultural sector in the manufacturing of inorganic fertilizers, as an oxidizing agent in explosive production as well as in the glass manufacturing industries when purified potassium nitrate is used (Ontumbi, Obando & Ondieki 2015). Nitrate in water can be as a result of natural or anthropogenic activities; atmospheric nitrogen fixation, rainfall and dry fall out, septic, animal waste, nitrate deposit, soil nitrogen, manure and compost, fertilizer nitrogen, plant residues, green manure as well as industrial effluent (Ternamche, 1991, Aksever, Davraz & Karaguzel, 2014).

KEBS (2015) has prescribed 10mg/L as the limit acceptable for nitrates in drinking water. The health problems associated with amounts above these levels are yet not well understood. Pennino *et al.* (2017) notes, that nitrate is a hazardous pollutant at high concentrations and is the most common contaminant of underground water and aquifers. Above Maximum Concentration Levels (MCLs) nitrate can be fatal to infants. It causes fatal methemoglobinemia (a blood disorder) to children less than six months. It is associated with the blue baby syndrome; a condition associated with reduced oxygen-carrying capacity in the blood. Further, it results in the formation of a carcinogenic agent known as nitrosamines, upon reaching the stomach or the liver (Pennino *et al.*, 2017). Given that some sources of nitrates are animal wastes and filtrates from septic and latrine linkages, this may indicate presences of microorganisms such as bacteria, protozoa, fungi and thus diseases and infections related or caused by these microbes.

2.4.4 Sulfate (SO₄²⁻)

Sulfate is also known as sulphate and is a naturally occurring mineral containing sulphur and oxygen. It is usually present in several salts, including calcium, magnesium, barium, potassium, and sodium. Sulfates are found in most soils and rocks and may be introduced into water as it percolates through soils and rocks. Sodium sulfates, magnesium sulfates, and potassium sulfates are all soluble in water, while calcium and barium sulfates are insoluble. Sulfates may be introduced into underground water through anthropogenic as well as natural activities, including animal matter, decaying plants, and through chemical products such as ammonium sulfate fertilizers. Water treatment plants that use aluminum sulfate and/or copper sulfate introduces sulfates into downstream water. Combustion of fossil fuels releases sulphur oxides to the atmosphere, which may

be converted to sulfate. It is a less toxic anion; however, consumption of high levels of sulfates, especially sodium and magnesium, may lead to diarrhea and intestinal discomfort resulting in dehydration. This is common when one takes water containing over 500mg/L WHO (2017). KEBS (2015), gives a guideline of 250mg/L as the permissible limit.

2.4.5 Manganese (Mn^{2+})

Manganese is a transition metal element that can or exists in several oxidation states. Mn (II) is the most common state of manganese that is present in water, it is water-soluble and bioavailable. On the other hand, Mn (IV) is not common in water and is essentially insoluble. According to the Federal-Provincial-Territorial Committee on Drinking Water (2016), manganese occurs naturally in the environment and is readily found in water, air, and soil. In underground water, manganese can emanate from the soil and rock weathering; it may also be due to anthropogenic activities that include industrial discharge, mining, and landfill leaching. This metal is used in different industries to manufacture an array of products, including steel, dry-cell batteries, fungicides, fertilizers, paints, and cosmetics. Manganese as permanganate is used as an oxidizing agent in water treatment plants. Manganese deficiency has been associated with poor lipids and carbohydrate metabolism as well as skeletal abnormalities. When taken in excess, manganese can cause several neuro-related complications. It has been reported to cause lethargy, mental disturbances, and increased muscle tone and tremor (Center for Diseases Control, 2019). KEBS (2015) recommends a limit of 0.4mg/L in drinking water.

2.4.6 Phosphates

Phosphorus is identified as being one of the most integral mineral elements necessary for nourishment of both for plant and animal life (Oram, 2021). Notably, Phosphates are formed from this mineral element. In the human body, Phosphorus significantly contributes in the formation of cell membrane structures. In water, Phosphates can either be formed as a particulate or a dissolved phase.

Phosphates are non-toxic to animals and human except when their concentrations in water are extremely high. For instance, in human bodies, extremely high concentration levels of phosphate ions could result in digestive problems. Conversely, Phosphate ions in very high concentration levels in a water source above the maximum the tolerable rate results in increase eutrophication of a water source that significantly impact water life (Oram, 2021).

There are certain recommended and permissible limits of Phosphate ions in water designated for a water source. The U.S. Environmental Protection Agency (EPA) advocates a certain endorsed criteria to establish on the recommended phosphorus concentration limit level in a water source. The regulatory body recommends a permissible Maximum Contaminant Level (MCL) of not greater than 0.1 mg/L for Phosphate ions in water to limit on accelerated eutrophication and accelerated growth in water life (EPA, 2018).

2.4.7 Iron

Iron is also another vital mineral element that can be found in water sources. Predominantly accounting for a significant percentage of the earth's crust, Iron is

registered as being one of the earth's natural resources that is in plenty (Mwaniki *et al.*, 2013). The Iron content in water is as a result of groundwater infiltrating the underlying geologic formations rich in Iron; hence the Iron is able to dissolve into the water making it a rich source of Iron.

Beneficial outcomes of drinking Iron-rich water include regulating body fluids and hormones, boosting and strengthening the immune system activity, regulating body temperature, prevention or treatment of anemia, boosting the gastrointestinal processes, enhancing energy levels in the body, and improving cognitive development and growth in young children.

Despite high concentration levels of Iron in water registered as not posing any adverse health effects on human health, there is certain recommended criterion that has been set for Iron concentration limit by the American regulatory body (Mwaniki *et al.*, 2013). The U.S. Environmental Protection Agency (EPA) does not contemplate heightened levels of Iron Concentrations to be an hazardous concern to human health, however the regulatory body has set the maximum contaminant level (MCL) of Iron in water at a limit of 0.3 mg/L (EPA, 2018).

2.4.8 Potassium

Potassium is a crucial mineral element for human life and is hardly, if ever, present in water in concentrations that could be deemed a concern for human health (WHO, 2017). Potassium is a common element in the environment and as such is often present in water. For human health, increased intakes of Potassium content is associated with a decrease in

blood pressure and in helping protect against cardiovascular conditions (Mwaniki *et al.*, 2013).

Even though excessive intake of potassium might induce a laxative effect, the American regulatory body is yet to recommend an Official Maximum Contaminant Level (MCL) for Potassium in water. This is attributed to the fact potassium concentrations in water are generally in smaller concentration levels, hence it is deemed unnecessary by the U.S. Environmental Protection Agency to set a health-based guideline value for potassium concentration in water (U.S. Environmental Protection Agency, 2018). WHO and KEBS have also not set health-based guideline for this element in water, (Oyem, Oyem & Ezeweali, 2014).

2.5 Microbial analysis

2.5.1 Indicator Organisms

These are microorganisms such as viruses and bacteria found in water and are used to determine the presence of pathogens in water (Amir and Zhengjian, 2019). Indicator organisms are very useful in determining the quality of water and are used for several purposes; as indicators of fecal pollution, used in assessing the effectiveness of water treatment processes like disinfection and filtration and in determining the cleanliness and the integrity of distribution systems in what is known as operational monitoring. These organisms include fecal coliforms such as *Escherichia coli*, fecal Streptococci, and Bacteriophage (Amir and Zhengjian, 2019).

2.5.2 Total coliform bacteria

This encompasses a wide range of bacteria, including aerobic and facultative, anaerobic, gram-negative and non-spore-forming bacilli that can tolerate fairly high concentrations of bile salts with the fermentation of lactose and production of aldehyde or acid at a temperature of 35-37 °C within a span of 24 hours. This group includes both environmental and fecal species. Coliforms can be found in sewage as well as in natural waters. Most of these bacteria emanate from the fecal matter of both human and livestock; given that these are heterotrophic bacteria, they readily multiply in soil and water environments. They as well survive, grow and multiply in water distribution systems, essentially in the presence of biofilms. KEBS (2015) prescribes that coliforms must be absent in drinking water.

2.5.3 Fecal coliforms

This is a subgroup of total coliforms present only in the guts and in the feces of warm-blooded animals. Owing to their origin, fecal coliforms are considered better contaminant indicators than total coliform bacteria. WHO and KEBS recommend zero detection of these bacteria in drinking water.

2.6 Carbonation

2.6.1 Definition of carbonated water and brands of carbonated water

Carbonated water is as well commonly christened soda water, fizzy water, or bubbly water. Water is described as being carbonated if carbon (IV) oxide has been dissolved under pressure, according to Steen and Ashurst (2006). The pressure may be from

technological innovations or naturally occurring geologic sources such as some water springs. There are varied types of water that have already been carbonated.

The types include soda water, tonic water, sparkling water, mineral water as well as club soda. Characteristically, tonic water, soda water, club soda, as well as mineral water, contain numerous, dissolved minerals. The minerals bolster their flavours greatly. Such minerals may include sodium bicarbonate, quinine, potassium sulfate, and potassium bicarbonate. The only additive in sparkling water is pressurized carbon (IV) oxide according to Steen and Ashurst (2006).

Carbonation processes make water effervescent. One can carbonate water at home using soda makers. Carbonated water meant for the market is usually packed in cans or bottles. Regardless of whether soda water is carbonated at home or in factories, it is comparable to basic carbonated water. At times, such soda water contains various minerals, which are dependent on who bottles the water. The additive minerals may include disodium phosphate, potassium sulfate, potassium citrate, sodium citrate, table salt, potassium bicarbonate, or sodium bicarbonate according to Steen and Ashurst (2006).

Such minerals are dissolved in water to make it taste somewhat salty like the traditional soda water. The water from Kiplolok Bubbling Springs being carbonated means that there are processes, which are naturally occurring, at the springs that give rise to effervescent mineral water (Steen & Ashurst, 2006). There are various artesian wells across the world producing carbonated water; for instance, the Selters artesian wells in German have been described rather comprehensively by Lennert (2003) and the Mihalkovo artesian wells in Bulgaria.

There is a wide variety of soda water in Kenya going by varied brand names. Highlands Mineral Water (2018) has a wide range of flavoured carbonated water in the market under the Club Soda brand name. Schweppes soda water, which Jacob Scheweppe introduced in the 1700s, is available in the Kenyan carbonated water market. Scheweppe produced the water through the displacement of water with specified amounts of carbonic acid, according to Buy Drinks Kenya (2018).

Other brands of carbonated water in the local market include Keringet, Kabarnet, San Pellegrino, and Aquamist (FoodPlus, 2018). The Aquamist brand of carbonated water is drawn from water springs within the Great Rift Valley and is processed and packaged at the source.

2.6.2 History of carbonated water

Traditionally, multiple alcoholic beverages, including champagne and sparkling wine, have been carbonated by fermenting them. There is a broad consensus that William Brownrigg was the first person to carbonate water artificially in the first half of the 18th century. The carbon (IV) oxide used by Brownrigg in carbonating the water was drawn from mines. Gabriel François Venel of France carbonated water artificially in mid-1750. Dr. Macbride of Ireland carbonated water artificially in 1764: when he was studying the putrefaction process, as well as fermentation (Schwartz, 2018).

Henry Cavendish developed equipment for carbonating water in 1766 and analyzed mineral water samples drawn from a local source. The development of the equipment motivated Joseph Priestley to execute extensive researches into carbonated water according to (Schwartz, 2018). A year later, Priestley determined that one could

carbonate water by suspending water vessels over beer vat (Schwartz, 2018). After suspending water over the vat, the water gained a pleasurable taste.

Priestley covered lots of research ground that gave way to the carbonation of water using sodium bicarbonate. Notably, the uses of sodium bicarbonate then included its use in producing drugs and producing baking powder. During that time, sodium bicarbonate was obtained by bubbling carbon (IV) oxide through plant ashes, which contain sodium carbonate typically (Jungnickel & McCormack, 1996).

In the early 1770s, Priestley published the outcomes of a research study in which he mixed chalk with sulfuric acid to generate carbon (IV) oxide and dissolved the carbon (IV) oxide in water to carbonate it, forming soda water (LaMoreaux & Tanner, 2001; Schils, 2012). In the 1780s, several companies that produced soda water on a large scale, including the then Manchester-based Thomas Henry Company and Schweppe, set their operations based on enhanced versions of the apparatus developed priorly by Priestley (LaMoreaux & Tanner, 2001). Notably, Priestley is widely acknowledged as being the carbonated water industry's founder. Priestley set up a carbonated water business in London in 1792 as submitted by (Schwartz, 2018).

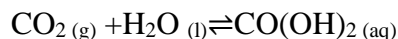
Seven years later, Augustine Thwaites set up his soda water company in Dublin. The company was the first business to patent the Soda Water brand name and use it in selling its carbonated water products. It is largely thought that the brand name was conceptualized by Richard Bewley, who was a Norfolk-based chemist. Bewley observed that whenever he added even a minute quantity of sodium carbonate into the water he

bolstered the rate at which the water absorbed carbon (IV) oxide greatly (LaMoreaux & Tanner, 2001).

Presently, water is artificially carbonated through the bubbling of pressurized carbon (IV) oxide through it in factories. Pressure bolsters the rate at which the water absorbs carbon (IV) oxide greatly. The pressure makes water absorb more carbon (IV) oxide than it would at the STP (Standard Atmospheric Pressure). When one opens a bottle carrying carbonated water, he or she releases the held pressure as carbon (IV) oxide exits the water, forming the typical bubbles.

2.6.3 Chemistry of Carbonated Water

When one dissolves carbon (IV) oxide (CO_2) in water, especially at a concentration of between 0.2% and 1%, carbonic acid (OC(OH)_2) is formed in a reaction that is reversible (Steen & Ashurst, 2006).



The carbonic acid is what makes carbonated water have a flavor that is somewhat sharp-tasting. It gives the water a pH ranging between 3.0 and 4.0. Notably, that pH level is less than the human stomach's pH level. Human beings forcefully maintain the desirable pH levels in their bodies through robust base-acid homeostasis (Steen & Ashurst, 2006). That means that the bodies are not substantially impacted by the intake of water laden with low levels of carbonic acid or carbonated water. The addition of alkaline solutions to carbonated water enhances the water's pH levels, decimating its acidity accordingly.

The amount of carbon (IV) oxide that water can absorb is determined using Henry's Law. To ensure that water absorbs the greatest possible amounts of carbon (IV) oxide, it is first chilled to temperatures that are just above the water's freezing temperature (Steen & Ashurst, 2006). More carbon (IV) oxide dissolves in water when it is at high pressure, and the water's temperature is low than when it is at low pressure, and the water's temperature is high. If the temperature is enhanced or the pressure is decimated, the dissolved carbon (IV) oxide effervesces to the atmosphere as bubbles.

2.6.4 Benefits to Human Health

The present body of literature relating to water consumption shows that the usage of carbonated water may present the human body with varied health-related benefits and several ill-effects. Various studies have demonstrated that carbonated water causes dental erosion (Catriona *et al.*, 2007; Damle, Bector & Saini, 2011). In their study, Catriona *et al.* (2007) concluded that flavoured carbonated water causes erosion.

Catriona *et al.* (2007) advised that water should be considered as possibly acidic beverage instead of being taken as water with specific flavouring. Damle, Bector, and Saini (2011) appraised the oral wellbeing of numerous children who were using carbonated drinks and sought to establish why the drinks are excessively consumed. They established that children who use carbonated drinks often have an increased possibility of suffering tooth decay.

There are widespread fears that the use of carbonated water is closely associated with the exacerbation of Gastro-Oesophageal Reflux Disease (GERD). That is because of the water's carbonation and low pH levels (Cuomo *et al.*, 2009; Johnson *et al.*, 2009). Even

then, Johnson *et al.* (2009) concluded that there is no clear proof that carbonated water exacerbates or even promotes GERD. Cuomo *et al.* (2009) submitted that there is no clear proof that carbonated water and the carbon (IV) oxide bearly affect the lower digestive tract's pathophysiology given that the gas is just about absorbed before getting to the tract.

Rather, Cuomo *et al.* (2009) held that carbonated water helps the body in several ways, especially in relation to the regulation of particular body functions. First, it helps the human body in maintaining a favorable base-acid equilibrium in the blood. Second, it helps exert the requisite tonic impact on one's peripheral circulation as well as blood circulation in the heart. Third, it helps in controlling respiration.

According to some studies referred to by Cuomo *et al.* (2009), carbonated water bolsters amylase activation in human saliva and pancreatic juice activation, especially due to carbonic acid ionization bicarbonate, as well as hydrogen ions. According to some of the studies, the utilization of carbonated water bolsters gall bladder activity. It increases the gall bladder's contraction efficiency.

2.6.5 Packaging of Carbonated Water

There has been marked growth in carbonated drinks' consumption in recent times (Ashurst, 1995). There is widespread thinking that technological advancements have increased consumption, especially in relation to the growing production of plastic and glass bottles and cans and the advent of speedy packaging systems and lines, according to Ashurst (1995), Ghose and Nair (2013).

Notably, companies that package carbonated water in plastic and glass containers need varied equipment configurations together with specialized technicians. For instance, when transferring carbonated water from a large storage tank into a bottle, the bottle should be sealed first and then pressurized, according to Ghose and Nair (2013). The most basic of the equipment configurations enables counter-pressure fillers to pressurize the bottle with carbon (IV) oxide to the pressure level of the storage tank.

Consequently, it fills the bottle with the water before relieving the pressure systematically. Lastly, the bottle is capped. There are other equipment configurations. The commonest of equipment used in carbonated water packaging include palletizer, date coder, warmer, and re-crater. Others include crater, carton erector, carton sealer, labeler, and shrink packer (Ghose & Nair, 2013).

Most of the packaging used in selling carbonated water is used in selling it to consumers just once. Consequently, repeated buying by the consumers is subject to the carbonated water in the packaging (Ashurst, 1995). Even then, if the packaging does not present the consumers with the anticipated functionality, they can easily purchase alternative carbonated water brands regardless of the related product qualities (Ghose and Nair, 2013).

2.6.6 Statistics on Consumption of Carbonated Water

The present body of literature shows that the consumption of carbonated water has increased worldwide in recent times (BMI Research, 2016; Masese, 2014; Nielsen, 2018; Statista, 2018). The increased consumption is especially evidenced by the growing bottled water sales across the world. Notably, the bottled water market consists of both

the carbonated water and non-carbonated water. The total bottled water revenue realized in Europe from the beginning of 2018 to September 21, 2018 was US\$20,375 million, according to Statista (2018).

The total bottled water revenue realized in China from the beginning of 2018 to September 21, 2018 was US\$42,774 million. The market is expected to register an annual growth of 2.4% across Europe going forward, according to Statista (2018). The total sparkling water revenue realized in the US from the beginning of 2018 to August 18, 2018 was US\$49 million, representing an increase of 22% from the figures registered within the corresponding period in 2017. The market for sparkling water in the US for the four year-period ending August 18, 2018 grew by 54%, according to Nielsen (2018).

In 2016, BMI Research released a report forecasting that the Tanzanian bottled water sales would grow by at 6.3% between then and 2020 (BMI Research, 2016). Masese (2014) references a Euromonitor report published in July 2013. The report projected that the Kenyan bottled water sales volume would grow robustly due to Kenyans' growing health consciousness and the poor quality associated with tap water in the country. By 2014, Kenya already had at least 80 lawfully operating water bottling companies. According to the report, the water bottling firm with the biggest share of the Kenyan bottled water market was Crown Foods at 23% share of the market (Masese, 2014).

2.7 Physico-Chemical parameters

2.7.1 Electrical Conductivity

The electrical conductivity (EC) of water is a water quality parameter that measures the water's ability to carry or conduct an electrical current by assessing the purity of water

depending on the presence and concentration of ions. The electrical conductivity (EC) of water increases as the concentration of ions also increases in the water. It is one of the main water quality parameters that assess the suitability of water for human purposes, such as irrigation and firefighting (Omer, 2019).

According to the World Health Organization (WHO, 2017), drinking water quality standards under electrical conductivity the recommended range value should not exceed 400 $\mu\text{S}/\text{cm}$ for drinking water standards (Meride & Ayenew, 2016). Moreover, the electrical conductivity (EC) of water is also impacted by the water temperature level, whereby the warmer the water, the higher the water conductivity would be.

2.7.2 Turbidity

Turbidity refers to the clarity, clearness or cloudiness of water, and it is an essential parameter in water quality (Damyanova, Ivanova & Yocheva, 2016). The turbidity is dependent on the quantity of solid matter found around its water catchment area. It is as a result of suspended material including organic material, clay, plankton, silt, and other particulate materials in water (Omer, 2019).

According to the World Health Organization (WHO) drinking water quality standards, the WHO recommended turbidity value of drinking water should not be more than 5.00 NTU and should ideally be below 1.00 NTU. Turbidity should ideally be kept below 1.00 NTU because of the perceived effects on disinfection (World Health Organization, 2017). Turbidity is an integral parameter because it can affect the taste and odor of drinking water. Thus, it is crucial and necessary to reduce the turbidity of water to effectively disinfect it. Turbidity could act as a shield to pathogens, and the particles that cause

turbidity can harbour bacteria and viruses (Omer, 2019). Groundwater has typically lower turbidity due to natural filtration of the water as it penetrates through the soil

2.7.3 Total Dissolved Solids

Water can dissolve a wide range of minerals, ions, salts or metals such as chlorides, potassium, calcium, magnesium, bicarbonates, sodium, and sulfates. These minerals produced an unwanted taste and diluted colour in the appearance of water. Water with a high TDS value shows that water is greatly mineralized (Oyem, Oyem & Ezeweali, 2014).

Generally, high TDS levels in groundwater are not harmful in everyday domestic use. However, these high concentrations are dangerous for individuals suffering from kidney and heart diseases. Water high levels of TDS might also cause laxative or constipation effects. According to the World Health Organization (WHO) drinking water quality standards, the maximum desirable limit for Total Dissolved Solids is 500 mg/L. The maximum permissible limit in the absence of a better source of water is 2000 mg/L for drinking purposes (World Health Organization, 2017).

2.7.4 Total Hardness

Water hardness is an indicator of the water reaction with soap. Hard water tends to form more precipitates when it reacts with soap. On the other hand, Total Hardness (TH) is a water quality parameter used to assess the dissolved minerals' effect, calcium and magnesium, to determine the appropriateness of the water for diverse domestic purposes drinking. Calcium and magnesium mineral ions cause the most significant hardness in naturally occurring waters. Calcium and magnesium presences in water is attributed to

percolation of rock and soils containing these elements especially limestone deposits. The dissolved minerals in water often cause potential issues such as scale deposits in hot water pipes and difficulty producing lather with soap (Omer, 2019; Shams et al., 2019).

Hardness is most commonly expressed as milligrams of calcium carbonate equivalent per litre. According to the World Health Organization (WHO) drinking water quality standards, water containing calcium carbonate at concentrations below 60 mg/L is generally considered as soft; 60–120 mg/L, moderately hard; 120–180 mg/L, hard; and more than 180 mg/L, very hard (World Health Organization, 2017). Hardness up to 300 mg/L is generally considered safe for consumption. However should the hardness level exceed this amount then the water might cause a laxative effect.

2.7.5 Water Temperature

Temperature is a key parameter because it influences water chemistry. Palatability, viscosity, solubility, odours, and chemical reactions of water are influenced by temperature. There is no specified guideline for temperature in water used for drinking and domestic purposes. However, relatively high temperature negatively impacts water quality by enhancing micro-organisms, which may increase taste, odor, color, and corrosion problems. Therefore, it is vital for groundwater temperature not to be too high to not have microbial proliferation (Omer, 2019).

Temperature affects biological, chemical and physical activities in the water. Besides, high temperature also decreases dissolved oxygen and increases electrical conductivity of water. Groundwater temperature is considered ambient and more palatable than warm water for safe drinking. The World Health Organization (WHO) drinking water quality

guidelines recommend a permissible water temperature limit between 25 - 30 °C (WHO, 2017).

2.7.6 Water pH

Water pH is one of the most integral parameters of water quality. It is used to indicate the strength of acidity or basicity of water solution (Oyem, Oyem & Ezeweali, 2014). In other words, pH of water is a measure of how acidic or basic water is. Water pH is really a measure of the relative amount of free hydrogen and hydroxyl ions in the water.

According to the World Health Organization (WHO) drinking water quality standards, the WHO recommends a maximum permissible limit of water pH from 6.5 to 8.5. This maximum permissible limit of water pH is considered as a safe range for water to be used for domestic use (WHO, 2017).

2.8 Related studies

A study by Mwaniki *et al.*, 2013, on the physicochemical properties of the spring water in Kabare and Baragwi in Kiranyaga County was comparable to this study. Like waters in Kiplolok bubbling springs in Nandi, the water in Kabare and Baragwi springs was acidic with a pH value of 6.48. It contained iron and manganese concentrations of 0.6762mg/L and 0.2972mg/L, respectively. However, this study did not determine the concentration of dissolved carbon dioxide.

Another study conducted to assess the microbiological quality of natural spring waters in Ebonyi and Enugu state in southeast Nigeria established that water from the sampled points was contaminated with bacteria. Some of the bacteria detected in these springs included *Pseudomonas* spp, *Escherichia coli*, *Vibrio* spp, *Staphylococcus* spp, among

others (Agahet *al.*, 2018). Similar results were reported for Kiplolok Bubbling spring water where Total and faecal coliforms were detected.

In 2011 Elisha conducted a study comparing water from different water sources and at different hydrological cycles in Isiolo, Kenya. The research showed that the physicochemical properties significantly differed ($p \leq 0.05$) across different water sources while there was no significant difference between different weather seasons.

2.9 Analytical techniques

2.9.1 Flame photometry and the working principle

Flame photometers work by measuring the intensity of radiation emitted by gaseous metallic atoms when atomized. As such the wavelength of the color emitted is a unique characteristic of a specific element and its intensity determines the concentration of that element in the sample under determination. Thus, the intensity is directly proportional to the concentration of analytes in the solution under aspiration. When a solution containing the compound of the metal under determination is aspirated into a flame, the solvent evaporates leaving a solid residue. The residue is then vaporized and dissociate into free gaseous atoms in the ground state. However, some of atoms absorb energy flame and are raised to higher excited electronic state, and radiate energy as they fall back to ground state. Filters are then used to isolate the required spectral emission; the intensity of this spectrum is then measured by a photosensitive detector coupled to an amplifier.

2.9.2 Ultraviolet/Visible Absorption Spectroscopy

Molecules with non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet and/or visible light. These electrons get excited to higher anti-bonding molecular orbitals. Some of the possible transitions involves electrons moving from the

highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). However, other transitions can as well be observed resulting to a number of absorption bands (figure 2.1) (Subodh, 2006).

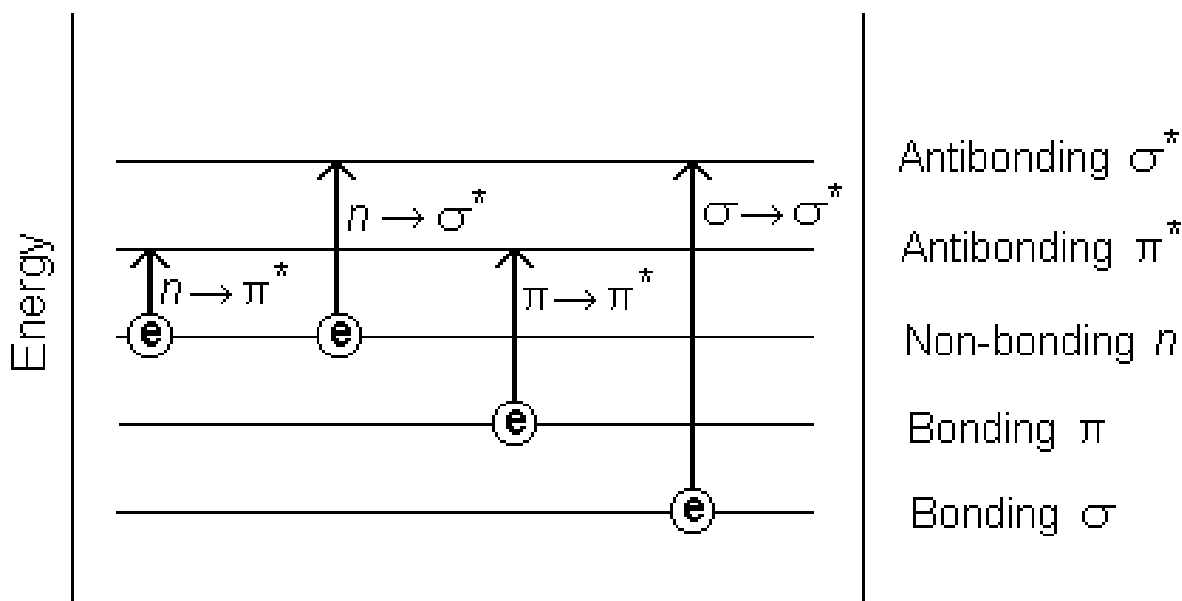


Figure 2. 1: Relative Energies of Orbitals Electronic Spectroscopy of Organic Molecules. Source Skoog et al., 2004

Before changing the excitation state, an atom has to absorb sufficient amounts of radiation for electrons to move to higher molecular orbitals. Longer bandgaps correspond to absorption of longer wavelength of light. The radiations required for molecules to undergo transitions are thus electrochemically specific. UV-Vis spectrophotometer thus uses this principle to quantify these analytes in a sample under investigation based on their absorption characteristics (Electronic Spectra, 2015).

The technique thus measures the intensity of the light or radiation transmitted through a sample in comparison to the reference measurement of the incident light source. HACH DR6000 UV-Vis spectrophotometers from the HACH company uses pulsed Xenon flash

lamp to determine small amount of samples within the ultra violet and visible wavelength range of 190-840nm, and uses a CCD optical detector with a wavelength accuracy of 0.4nm Figure 2.2 (Hach, 2019). This method follows the Beer-Lambert Law which states that; the amount of light absorbed is directly proportional to the concentration of the sample and the path-length.



Figure 2. 2: Image of UV-Vis Spectrophotometer HACH DR6000 at ELDOWAS Laboratory

CHAPTER THREE

METHODOLOGY

3.1 Area of study

The springs are on a three-acre piece of land in Kipkolok village, Kilibwoni ward, Emgwen sub-county, Nandi County. It comprises several natural holes that have pulping salty lime water. Indeed according to the local community, this water tastes like sprite soda or tonic water, alluding to the fact that the bubbling of the water may be due to the high pressure of dissolved gases emanating from geological activities and processes. Below is figure 3.1 showing the map sampled area, with the sampled points indicated in the map



Figure 3. 1: Map of Kenya showing Nandi County. (Source : GoK, 2015)

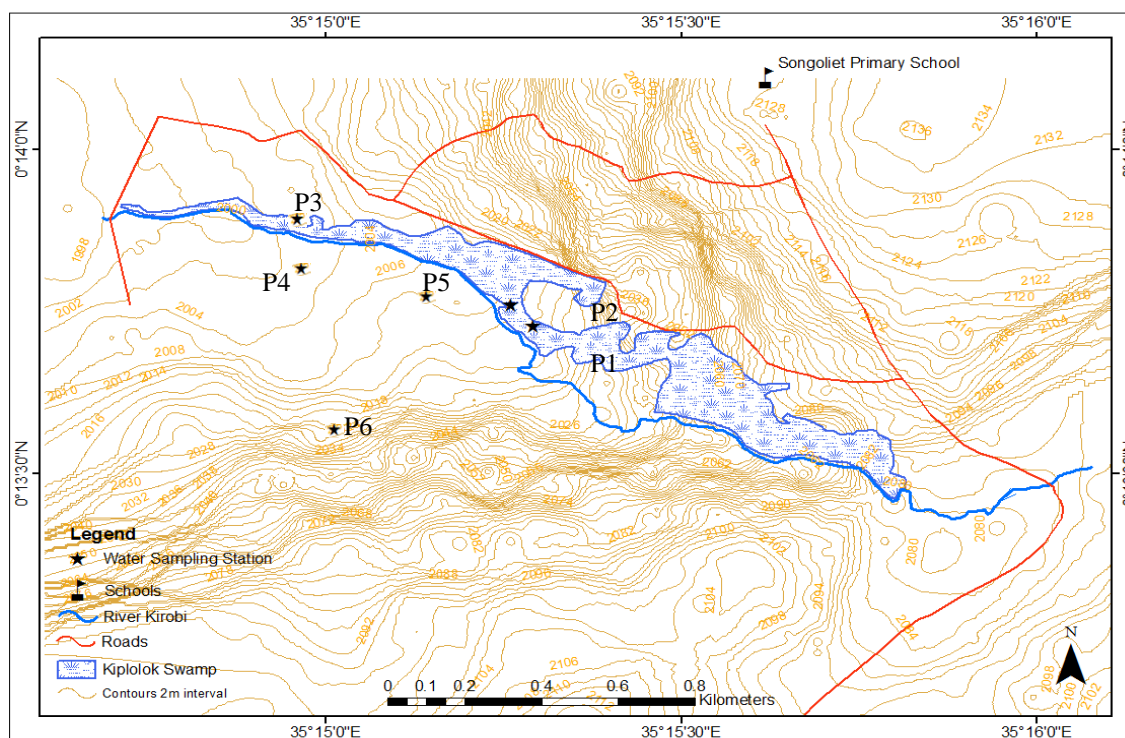


Figure 3. 2 Map of Kiplolok Area Showing the Sampled Sites.

(Source : Ng'etich, 2019)

3.2 Chemicals and reagents

All the reagents used in this study were of analytical grade. They included: Nitric acid (37%), Potassium dichromate, silver nitrate, sulphuric acid, barium perchloric (72%) anhydrous, isopropanol, ammonium molybdate tetrahydrate, ascorbic acid, ammonium persulphate, EDTA solution, Eriochrome black, Ammonium chloride, distilled water, sodium hydroxide pellets (purity 98%). The chemicals were bought from Reagent Chemical Services (UK), HACH (US) and Sigma Laboratories in the United States of America

3.3 Research design

This study used purposive sampling method. Six sampling sites from which the residents draw water were identified and sampled. These sampling sites included; three bubbling points (P1, P2, and P4), one shallow well (P3), a river (P5), and a spring (P6). These points were located using gps Garmin etrex 10 model. Sampling was done; during the rainy season in May and during the dry season in February. A total of 60 samples were collected, five from each sampling point from each season for cations and anions, sodium and potassium determination, microbial analysis, carbonation, and physicochemical analysis. The sampling containers were then properly labeled for easier identification and recording purposes. After the sampling most of the physico-chemical parameters such as pH, temperature, electrical conductivity, and Total dissolved solids were determined in situ. Analyses for the other parameters were done through laboratory analysis. All the samples were analyzed in triplicates and the standard deviations recorded. One way analysis of variance was then done on the results obtained to ascertain whether or not there were significance differences between data obtained in different seasons as well as that reported for different sampling sites.

3.4 Sample collection

3.4.1 Sample collection for Cations and Anions

100mL of the water samples were collected in 100mL plastic containers. At each sampling point, sampling containers (cleaned and rinsed with distilled water) were rinsed twice with the sample water prior to sample collection. 1 ml Nitric acid was added to samples collected for chemical analysis for preservation purposes. The containers were tightly sealed. These samples were then transported in cool boxes to Eldoret Water and

Sanitation (ELDOWAS) laboratory. Samples for determination of sodium and potassium were taken to University of Eldoret (UoE) Chemistry Laboratory for analysis.

3.4.2 Sample collection for microbial analysis

500mL of 12 samples 6 each for each season for microbial analysis were collected in 500 mL sterilized Schott Duran Bottles; the bottles had been thoroughly sterilized in the ELDOWAS laboratory prior to sample collection. Sterilization of the sample container was done by washing the container thoroughly using tap water and rinsed with de-ionised water, 5 drops of sodium thiosulfate was then added into the containers to remove any chlorine residue that might interfere with the sample. The containers were then corked properly, covered with aluminium foil and autoclaved for 20 minutes at a temperature of 120 °C. The samples were then kept in icebox and transported to Eldoret Water and Sanitation (ELDOWAS) laboratory for analysis within three hours to minimize microbes' growth.

3.4.3 Sample collection for dissolved carbon dioxide analysis

500mL of water samples were collected in 500mL plastic containers. A total of 12 samples were collected, 6 each for each season. The containers were tightly corked to minimize carbon dioxide loss and transported to the KEBS Laboratories in Eldoret within three hours for analysis.

3.4.4 Sample collection for physico-chemical analysis

The water temperature, electrical conductivity, total dissolved solids (TDS), were measured in situ, using portable pre-calibrated HANNA waterproof Tester pH, TDS, Temperature, and Electrical Conductivity Meter (model HI 98129), as soon as the

samples are collected. The instrumental was first set to zero reading using de-ionised water before taking the sample readings.

3.5 Preparation of standard solution

3.5.1 Preparation of Potassium and Sodium Standards

0.10 M stock solution of potassium and sodium were prepared by dissolving the correct amount of analytical grade potassium chloride as well as sodium chloride salts in deionized water. Working standard solutions for the metals ranging from 1ppm to 10ppm were prepared by dilution of the stock solution accordingly and were used to prepare sodium and potassium calibration curves (Appendices I and II).

3.5.2 Preparation of EDTA standard solution

EDTA of analytical grade was dried at 80⁰C to remove moisture. 9.306g of the dried EDTA was then dissolved in 250ml of deionized water, making 0.10 M of EDTA standard solution.

3.6 Laboratory analysis

3.6.1 Cations and Anions

3.6.1.1 Determination of Manganese

The concentration of manganese ions in the water samples was determined using spectrophotometer -DR6000 HACH model using periodate oxidation method. The analysis was done under Program 295 manganese on the DR6000 HACH model spectrophotometer. Prior to the actual analysis 5 N sodium hydroxide standard solution was added to the sample to adjust the pH to 4-5.

In determining manganese concentration in the water sample, the sample cell was filled with 10mL of the water sample. One buffer powder pillow, citrate type content was added into the sample and thoroughly mixed. Sodium periodate was added to the sample cell and inverted to mix thoroughly. After the mixing, a violet colour appeared, indicating the presence of manganese. The prepared sample was then allowed 2 minutes reaction time. A blank sample cell was then used to calibrate the instrument. The sample cell was then inserted into the instrument and the reading recording in mg/L Mn. The measurement was done at 525 nm wavelength.

3.6.1.2 Determination of Total Iron

The concentration of total iron in water samples was done using the spectrophotometry method (Hach DR6000 spectrophotometer model was used.) Hach Method 8008 was used. Before the analysis, samples with a pH of below 3-5 were adjusted to this range using 5 N sodium Hydroxide solution. The appropriate program for analysis of total iron using Hach method 8008 was selected (265 Iron, FerroVer program) on the instrument, and the analysis was done at 510nm. 10 mL of the sample was measured into a 10 mL sample cell. The contents of one FerroVer Iron reagent powder pillow (a solution of thioglycolic acid and ammonia) were added to the sample cell. Mixing of the sample was done by swirling the sample cell. The mixed content was given a 3 minute reaction time. Calibration of the instrument was done by inserting a blank cell holder into the instrument and setting the instrument at zero reading. The sample cell with the sample was then measured and the reading recorded.

3.6.1.3 Sodium and Potassium

Flame emission photometry was used to determine the concentration of both sodium and potassium. Pre-prepared standards of potassium and sodium were used to calibrate the Flame Photometer at a wavelength of 768 and 589 nm, respectively. After that, the concentration of the samples was deduced from the standard calibration curve.

3.6.1.4 Determination of Nitrate

The concentration of nitrate ions in the water samples was determined using spectrophotometer -DR6000 model using cadmium reduction method. For water samples with high turbidity filtration using 0.45micron filter paper was done before the actual analysis. 5 N sodium hydroxide solution was added to water samples to adjust the pH to 7 prior to starting the analysis. The DR6000 spectrophotometer was set, by selecting the appropriate program (355 N, Nitrate HR PP). 10 mL of the sample water was put into a sample cell then, one NitraVer 5 nitrate reagent powder pillow was added into the sample. This was then left standing for a 1-minute reaction time before putting the stopper on the sample cell and shaking the content to allow the undissolved powder to dissolve. A further 5-minute reaction time was set until amber color appeared, indicating nitrate presence. A blank of deionized water was put in a different cell sample and used to zero the instrument before the sample was inserted into the instrument. The concentration of nitrate was recorded at 500nm.

3.6.1.5 Determination of Phosphate

The concentration of phosphate ions in the water samples was determined using spectrophotometer -DR6000 model using Acid Persulfate digestion method. 5 N sodium

hydroxide solution was added to water samples to adjust the pH to 7 before starting the analysis. The DR6000 spectrophotometer was set by selecting the appropriate program (536 P, Total/AH PV TNT). 5 mL of the sample was added to total Phosphorus Test vial. The contents of one potassium Persulfate powder pillow were added. The vial was then capped, and the mixture was shaken vigorously to dissolve the powder. The vial was put into the reactor and allowed 30 minutes of reaction time. On the expiry of the 30 minutes reaction time, the vial was inserted into a 16 –mm cell holder, and the instrument reading was set to zero (0.00mg/L PO_4^{3-}). PhosVer 3 powder pillow was added to the vial, capped, and shaken for 20-30 seconds to allow proper mixing and a 2 minute reaction time allowed. The sample was then measured within 8 minutes after the 2 minute reaction time expired. The reading was then taken and recorded in mg/L PO_4^{3-} at 880nm wavelength.

3.6.1.6 Determination of Sulphate

Sulphate concentration was determined by DR6000 HACH spectrophotometer using Method 8051 Powder Pillows. DR6000 HACH program 680 Sulfate was used in this analysis. The sample was prepared in 4 steps. First, the sample cell was filled with 10 mL of the sample water. Secondly, SulfaVer 4 powder pillow was added to the sample cell. Thirdly, the mixture was mixed by swirling the sample cell; at this point, the water turned cloudy for samples that sulfate was present. Lastly, the sample cell was given 5- minute reaction time.

The blank was prepared by filling the second sample cell with 10 mL of the water sample. The cell was then wiped with a clean tissue and left to stand for 5 minutes then used to calibrate the instrument set to 0 mg/L SO_4^{2-}

The sample concentration was then taken by inserting the prepared sample into the cell holder and taking the reading in mg/L SO_4^{2-}

3.6.1.7 Determination of Chloride

50 ml of the water sample was measured and then diluted to 100ml. 1 ml of potassium chromate indicator solution was added into the sample and titrated with the standard silver nitrate solution until a pinkish yellow color appeared indicating the endpoint. The titration was done at a pH range of 7 to 10. The sample's pH were adjusted to this range by adding sodium hydroxide solution. The reagent blank was obtained by titrating 50ml of deionized water and 1ml potassium chromate against standard silver nitrate. The concentration of chloride ions in the water was then determined using the formula

Chloride was calculated using the equation 3.5. $\text{Cl}^- \text{ (mg/L)} = (A-B) \cdot N \cdot 35.450/\text{mL}$ of the water sample

Where A =Volume of titration sample (ml)

B = volume of titration of blank (ml)

N= normality of AgNO_3

3.6.2 Microbial analysis

3.6.2.1 Determination of Total coliforms and Feacal coliforms by Membrane Filtration

A 100mL of sample water was filtered through 47 mm, 0.45 μm pore size cellulose ester membrane filter, which retained the bacteria in the water sample. The filter was then

placed on a 5-mL plate containing MI agar, and the plate was then incubated at 35⁰C for 24 hours and the number of colonies determined.

3.6.3 Determination of Dissolved Carbon dioxide

Dissolved carbon dioxide was determined using a carbonation meter (CarboQC; Anton Paar GmbH, Graz, Austria). CarboQC is relatively small equipment weighing not more than 3.1 kg and measuring about 190*120*305 mm. It is used to measure pressurized carbon dioxide in liquids. The measuring chamber was filled with 10 ml of the sample to be determined. The chamber was then sealed, and the volume inside expanded twice by use of a motor-driven piston with an attached stirrer. The equilibrium pressure and temperature at every expansion step were determined and based on the difference in relative solubilities of dissolved carbon dioxide and the air-based in aqueous solution their net contribution was estimated. The calculated results were then displayed in about a minute and a half showing the amount of dissolved CO₂ in g/L.

3.6.4 Physico-Chemical Properties Analysis

3.6.4.1 Determination of Temperature, Electrical conductivity, TDS, and pH

Determination of Electrical conductivity, temperature, total dissolved solids (TDS), and the pH was done in situ using a portable pre-calibrated HANNA waterproof Tester pH, TDS, Temperature and electrical conductivity Meter (Model HI 98129). The meter was washed and rinsed with deionized water before it was inserted in the water sample and the reading recorded.

3.6.4.2 Turbidity determination

Turbidity determination was done by the nephelometric method. This method compares the intensity of light scattered by a sample to that scattered by a standard reference under defined conditions. First deionized water was used to standardize turbid meter (PAQUALAB) to zero, then to 80NTU, and finally to 40 NTU standard. The water sample was well mixed, transferred into a cuvette. The turbid meter readings were then recorded.

3.6.4.3 Determination of total hardness

50.0 mL burette was first rinsed with deionized water and EDTA solution to ensure it was clean. The burette was filled to 0.00mL mark with EDTA solution. 10.0mL pipette was flushed with the sample water and used to transfer 10.0mL of the water into a beaker. 5 mL of the ammonia-based pH 10 added into the water sample, followed by two drops of Eriochromschwartz –T indicator. The water sample colour turned to wine-red indicating the presence of calcium and magnesium ions. The water sample was then titrated against EDTA solution until a blue colour appeared, denoting the reaction endpoint.

The concentration of calcium and magnesium ions were then determined using the formula $\text{Conc}' (\text{EDTA}) * V_{(\text{av})} * 10^{-3} / V_{(\text{w})} * 10^{-3}$

CHAPTER FOUR

RESULTS

4.1 Introduction

The study was carried out in Kiplolok, Kilibwoni, Nandi County, Kenya. Samples were collected and analyzed for dry and wet seasons. Carbonation, chemical, microbial and physico-chemical properties of the samples were analyzed. One-way ANOVA was performed to determine whether there was a significant difference for the same parameters between the season and between sampling points.

Analytes concentrations for each parameter were compared with the WHO, KEBS, and some with the European Parliament Directive 2009/54/EC on mineral water. Parameters with higher values than the standards set by KEBS and WHO drinking water are shown in red in each table, while pH that was lower than those accepted by the KEBS and the WHO is shown in yellow. Important to note that KEBS and 2009/54/EC recommend a minimum pH value of 4 for carbonated water.

4.2 Cations and Anions

4.2.1 Iron and Manganese

Table 4. 1: Concentrations of Iron and Manganese during the rainy (RS) and dry (DS) seasons

Sampling Points	Iron (mg/L)		Manganese (mg/L)	
	RS	DS	RS	DS
P1	3.03 ±0.02	2.44 ±0.01	0.8 ±0.08	1.0 ±0.14
P2	4.02 ±0.02	3.86 ±0.01	0.8 ±0.05	0.6 ±0.08
P3	0.17 ±0.01	0.19 ±0.02	0.7 ±0.05	0.8 ±0.05
P4	1.21 ±0.02	0.72 ±0.08	1.2 ±0.05	0.9 ±0.05
P5	1.36 ±0.02	0.78 ± 0.01	0.4 ±0.08	0.5 ±0.09
P6	0.07 ±0.01	0.09 ±0.01	0.3 ±0.05	0.3 ±0.08
KEBS	0.3		0.5	
WHO	0.5		0.1	

4.2.2 Sodium and Potassium

Table 4. 2: Concentrations of sodium and potassium during the rainy (RS) and dry (DS) seasons

Sampling Points	Sodium (mg/L)		Potassium (mg/L)	
	RS	DS	RS	DS
P1	8.1 ±0.29	7.7 ±0.09	6.0 ±0.23	5.2 ±0.05
P2	6.8 ±0.08	7.7 ±0.16	3.4 ±0.05	4.0 ±0.12
P3	8.7 ±0.16	10.0 ±0.12	6.8 ±0.12	6.0 ±0.08
P4	7.4 ±0.05	7.2 ±0.08	4.0 ±0.05	3.8 ±0.03
P5	10.5 ±0.12	9.3 ±0.12	7.1 ±0.08	6.2 ±0.08
P6	6.2 ±0.08	5.8 ±0.05	4.6 ±0.05	4.0 ±0.08
KEBS	20		-	
WHO	20		-	

4.2.3 Nitrate, Phosphate, Sulphate and Chloride

Table 4. 3: Concentrations of Nitrate, Phosphate, Sulfate, and Chloride during the rainy (RS) and dry (DS) seasons

Sampling Points	Nitrate (mg/L)		Phosphate (mg/L)		Sulfate (mg/L)		Chloride (mg/L)	
	RS	DS	RS	DS	RS	DS	RS	DS
P1	1.0 ±0.05	1.8±0.05	0.51 ±0.014	0.56±0.005	1 ±0.5	0±0.0	4.004 ±0.0012	2.487±0.0005
P2	0.8 ±0.08	1.3±0.08	1.30 ±0.012	0.81±0.012	1 ±0.8	2 ±0.8	5.936 ±0.0005	6.978±0.0016
P3	6.3 ±0.05	5.1± 0.12	0.60 ±0.005	0.56±0.008	0 ±0.5	0±0.5	1.679 ±0.0014	1.499±0.0008
P4	0.0 ±0.00	0.1±0.05	0.96 ±0.008	0.85±0.016	1 ±0.5	1±0.8	3.112 ±0.0008	2.487±0.0012
P5	0.4 ±0.08	0.3±0.05	0.88 ±0.014	0.74±0.012	1 ±0.5	0±0.5	1.492 ±0.0016	0.999±0.0018
P6	1.8 ±0.12	1.6±0.05	0.92 ±0.005	0.74±0.008	0 ±0.5	0±0.0	0.2000 ±0.0002	0.499±0.0012
KEBS	50		45		400		250	
WHO	50		45		250		250	

4.3 Microbial results

4.3.1 Total Coliforms and Faecal Coliforms

Table 4. 4: Concentrations of Total and Faecal coliforms during the rainy (RS) and dry (DS) seasons

Sampling Points	Total coliforms (Cfu/100ml)		Faecal coliforms (Cfu/100ml)	
	RS	DS	RS	DS
P1	1180 ±4.71	1110 ±21.70	410 ±16.45	350 ±18.80
P2	1440 ±8.16	1410 ±17.21	400 ±10.31	440 ±21.32
P3	2400±18.12	2340 ±22.15	1180 ±4.71	1150 ±15.68
P4	1800 ±12.47	1840 ±12.47	900 ±22.54	850 ±8.61
P5	2300 ±21.60	2110 ±4.71	1110 ±19.14	910 ±17.29
P6	1170 ±16.23	1240 ±18.40	580 ±8.61	560 ±4.71
KEBS	Nil		Nil	
WHO	Nil		Nil	

4.4 Carbonation

4.4.1 Dissolved CO₂ and pH

Table 4. 5: Concentrations of Dissolved CO₂ and pH during the rainy (RS) and dry (DS) seasons

Sampling Points	pH		Dissolved CO ₂ (g/L)	
	RS	DS	RS	DS
P1	3.80 ±0.008	3.41 ±0.018	2.65 ±0.018	3.00 ±0.016
P2	3.90 ±0.014	3.56 ±0.025	2.50 ±0.008	2.80±0.037
P3	5.25 ±0.033	4.39 ±0.30	1.20±0.012	2.04 ±0.037
P4	4.00 ±0.045	4.10 ±0.052	1.98 ±0.016	2.55 ±0.041
P5	6.80 ±0.033	6.37 ±0.015	0.50 ±0.005	0.40 ±0.005
P6	4.78 ±0.041	4.93 ±0.027	1.60±0.013	1.50 ±0.012
KEBS	Minimum of 4		Minimum of 1.0 g/l	
2009/54/EC	Minimum of 4		Minimum of 1.0 g/l	

4.5 Physico-Chemical Parameters

4.5.1 Turbidity, Electrical Conductivity, Total Hardness, Total dissolved solids and Temperature

Table 4. 6: Turbidity, Temperature, Conductivity, TDS, and Total hardness during the rainy (RS) and dry (DS) seasons

Sampling Points	Turbidity (NTU)		Temperature (°C)		Conductivity (µS/Cm)		TDS (mg/L)		Total Hardness (mg/L)	
	RS	DS	RS	DS	RS	DS	RS	DS	RS	DS
P1	130.0 ±3.15	117.0 ±1.25	16.3 ±0.12	20.0 ±0.14	65 ±1.6	70 ±1.6	25 ±1.2	31 ±1.7	17.5 ±0.16	17.1 ±0.05
P2	290.0 ±4.70	261.0 ±4.50	15.7 ±0.05	19.9 ±0.08	60 ±2.1	65 ±3.7	24 ±0.5	30 ±0.5	17.6 ±0.08	17.5 ±0.12
P3	2.9 ±0.08	3.2 ±0.05	16.3 ±0.16	20.0 ±0.12	130 ±3.7	130 ±2.4	77 ±3.7	65 ±1.2	34.2 ±0.12	33.8±0.18
P4	320.0 ±5.40	224.0 ±5.25	15.8 ±0.18	19.0 ±0.18	70 ±0.5	66 ±1.8	27 ±1.2	34 ±1.7	18.2 ±0.05	17.1 ±0.05
P5	50.0 ±2.37	39.6 ±1.20	16.5 ±0.12	20.4 ±0.14	139 ±1.8	156 ±4.2	89 ±2.1	78 ±0.5	70.0 ±1.6	68.4 ±0.25
P6	4.7 ±0.16	3.3 ±0.12	16.6 ±0.18	20.7 ±0.17	55 ±0.8	51 ±3.1	28 ±0.8	25 ±1.2	17.2 ±0.17	17.7 ±0.08
KEBS	5				400		1200		300	
WHO	5				400		1500			

Table 4.7: GPS Coordinates for the Sites Sampled

Sampling Sites	GPS Coordinates
P1	36N 0750776 UTM 0025517
P2	36N 0750781 UTM 0025522
P3	36N 0750965 UTM 0025330
P4	36N 0750956 UTM 0025314
P5	36N 0750979 UTM 0025223
P6	36N 0750960 UTM 0025151

4.6 One Way anova statistical results

4.6.1 Iron

Single-factor ANOVA performed at alpha equals to 0.05 showed no significant difference between iron concentration in the rainy and dry season with a p-value of 0.744231 (Appendix 2a). On the other hand, there was a significant difference between different sampling points with a p-value of 5.3×10^{-5} (Appendix 2b).

4.6.2 Manganese

Single-factor ANOVA performed at alpha equals to 0.05 showed no significant difference between manganese concentration in the rainy and dry season with a p-value of 0.85831 (Appendix 3a). On the other hand, there was a significant difference between different sampling points with a p-value of 0.00435 (Appendix 3b).

4.6.3 Phosphates

According to one-way ANOVA there was no significant difference for the phosphates concentrations in the two seasons, which had a p-value of 0.363287 (Appendix 6a). There

was no significant difference between concentrations of phosphates recorded in different sampling points, reporting a p-value of 0.088847 (Appendix 6b).

4.6.4 Nitrates

One way ANOVA showed that there was no significant difference in nitrate concentrations report in the two seasons with a p-value of 0.989205 (Appendix 5a). However, there was a significant difference between concentrations recorded in different sampling with a p-value of 0.000134 (Appendix 5b).

4.6.5 Chlorides

There was no significant difference in the chloride concentration between the dry and the rainy season; ANOVA reported a p-value of 0.850503 (Appendix 8a). However, there was a significant difference in chloride between different sampling points with a p-value of 0.000485 (Appendix 8b).

4.6.6 Sulphate

As per ANOVA single-factor, there was no significant difference between the seasons concentrations nor any significant difference between different sampled points. With a p-value of 0.686744 and 0.126478 being recorded between seasons and points, respectively (Appendices 7a & 7b)

4.6.7 pH

Single-factor ANOVA showed that there was no significant difference in pH recorded in the two seasons with a p-value of 0.658148, but there was a significant difference in pH values between point with a p-value of 0.000677 (Appendix 9a and 9b)

4.6.8 Dissolved CO₂

Single-factor ANOVA showed that there was no difference of the concentrations of dissolved carbon dioxide recorded in the dry season and those in the rainy season with a p-value of 0.562903; on the other hand, there was a significant difference in concentrations reported between different sampling points with a p-value of 0.00261 (Appendix Xa and Xb).

CHAPTER FIVE

DISCUSSION

5.1 Cations and Anions

5.1.1 Iron and Manganese

Iron concentration in most sites were higher than the KEBS (2007) of 0.3 mg/L, and WHO (2015) of 0.5 mg/L maximum permitted limits in drinking water. Points with a concentration higher than the permitted limits included P1, P2, P4, and P5, as shown in Table 4.1. According to Jennings, (1964), Kapsebet and Kilibwoni Area under which Kiplolok falls under contain sedimentary rocks that are rich in iron. Hematite and magnetite rocks contain over 15% metallic iron (Kiptarus, Muumbo, Makokha and Kimutai, 2015)

Manganese concentration were reported to range from 0.3 ± 0.05 mg/L in sampling site P5 to 1.2 ± 0.05 mg/L for sampling site P4 during the rainy season while in the dry season the range was from 0.3 ± 0.08 mg/L in P6 to 1.0 ± 0.14 mg/L in P1 as shown in Table 4.1. These concentrations were higher than the maximum permitted limits of 0.1 mg/L (WHO, 2017). While P1, P2, P3, and P4, recorded higher concentrations than those permitted by KEBS of not more than 0.5 mg/L in both seasons, as shown in Table 4.1. This study therefore adopted the KEBS standards of 0.5 mg/L, as this is the statutory board mandated to maintaining the water quality in Kenya. High concentrations of both iron and manganese were attributable to the leaching of these minerals from soils and rocks as noted by Hassan, Ali, Abdel and Ashraf, (2019).

5.1.2 Sodium and Potassium

The sodium concentration in the sampled area conformed to the WHO and KEBS standards as shown in Table 4.2. Sodium in underground water is mainly due to erosion of salt deposits of sodium containing rock minerals (Christine, Kibet, Kiprop and Were, 2018). There was also no significance difference between the two hydrological periods of sampling, while there was significance difference on the concentration of sodium between points. This may indicate that rain or lack of it contribute insignificantly to the leaching of sodium into the water source. While the significant difference observed would be attributable to the diverse source of these water (Zizhao, Wenke, Ming, Zhitong, Chuan and Ying, 2020). For example water from River Kirobi is surface water while all there other sources were ground water.

Relatively low amount of potassium concentrations were reported in KBS water, the highest concentration being 7.1 ± 0.08 mg/L at sampling site P5 during the rainy season. According to WHO (2010), potassium is present in most natural sources of water but occurs in concentrations that are seldom, if ever, could be of concern to human health.

5.1.3 Phosphates, Nitrates, Sulfates and Chlorides

The phosphates concentrations were relatively low in these waters with sampling site P2 recording the highest concentration of 1.3 ± 0.012 mg/L shown in Table 4.3. Comparably all the concentrations were within the KEBS and WHO guidelines for drinking water of 45mg/L. Phosphates are naturally derived from the decomposition of organic matter as well as the leaching of phosphorus-rich agricultural inputs such as fertilizers and pesticides (Kumar, Kumar, & Prakash, 2019). Phosphates in the sampling points were

attributed to agricultural activities and the decomposing organic matter, especially the decaying swamp vegetation.

Nitrate concentrations from all the six sampling points during the dry and rainy season were all within the allowed WHO and KEBS limit of 50 mg/L. Sampling site P3 recorded the highest concentration of nitrate. The nitrate presence in water can be attributed to fertilizers leaching especially nitro-phosphorus fertilizers and human and animal waste (Christine *et al.*, 2018). That explains why high nitrate concentrations were recorded at P3 –the hand-dug well, located near cattle shed and a nearby pit latrine. High nitrate concentration in ground water may be an indication of contamination of such water, and this may be accompanied with high levels of bacteria concentration (Christine *et al.*, 2018). This findings were true for this study that recorded higher concentration of both nitrates and indicators microorganisms at sampling site P3.

Low concentrations of sulfate were recorded in all sampling points. The sulfate concentrations were recorded as zero in some points, indicating that sulfate was not detected during the analysis. Concentrations for the rainy seasons were 1.0 ± 0.5 mg/L for P1, P4, and P5, while sulfate was not detected in P3 and P6. During the dry season, sulfate was only detected in two sampling points P2 recorded 2.0 ± 0.8 mg/L, P4 recorded 1.0mg/L, while P1, P3, P5, and P6 sulfate were not detected shown in Table 4.3. KEBS standards for domestic drinking water sets for the maximum allowed sulfate concentration at 400 mg/L.

Chloride concentration in these waters ranged from 0.200 ± 0.0002 mg/L for P6 during the dry season and as high as 6.978 ± 0.0016 mg/L for P2 during the rainy season (Table 4.3). These concentration were within the ranged permissible for drinking water of 250

mg/L for WHO and KEBS. The area could be considered unpolluted especially industrial pollution, and therefore there is no industrial chloride contamination in the water. As note Ashun and Bansah (2017), in the absences of industrial chloride water contamination the other possible source of chloride would be anthropogenic, such as animal and human waste. This would be possible the case for the low chloride concentrations reported in this area.

5.2 Microbial

5.2.1 Total Coliforms and Faecal Coliforms

All the points sampled were contaminated with both total and faecal coliforms (Table 4.4). Total and faecal coliforms are as a result of human and animal waste contaminating water (Agahet *al.*, 2018). That explains why the hand dug well that is near a latrine and a cow shed reported the highest concentrations of total coliforms and faecal coliforms of 2400Cfu/100ml and 2340 during the rainy season and 1180 and 1150 during the dry season, respectively.

5.3 Carbonation

5.3.1 Dissolved CO₂

Dissolved Carbon dioxide concentrations ranged from 0.5 ± 0.005 g/L to 2.65 ± 0.018 g/L, during the rainy season and from 0.4 ± 0.005 g/L to 3.0 ± 0.016 g/L during the dry season (Table 4.5). The water samples from P1, P2, P3, P4, and P6 could be classified as carbonated based on the European Directive 2009/54/EC and 2003/40/EC that puts the minimum concentration of carbonated water at 1.0 g/L dissolved carbon dioxide (Table 4.5) (Agnew & Halihan 2017). However, the water sample from P5 (river) did not meet

the concentration requirement for carbonated water. Important to note is that the high concentration of carbon dioxide was as a result of underground water dissolved carbon dioxide released from the geological and volcanic processes. However, the water from the river being mostly surface water very little carbon dioxide emanating from these processes is dissolved. P5 (river) had the lowest concentration of dissolved carbon dioxide of 0.5 ± 0.005 g/L during the rainy season and 0.4 ± 0.005 g/L in the dry season. Additionally, the concentration of dissolved carbon dioxide had an inverse correlation with pH values.

5.4 Physico-Chemical Parameters

5.4.1 Turbidity, Temperature, Electrical Conductivity, pH, Total Hardness, TDS

Water pH is used to show the basicity or the acidity of the water. It is one of the most important water quality parameters (Oyem, Oyem & Ezeweali, 2014). Water pH is really a measure of the relative amount of free hydrogen and hydroxyl ions in the water. According to the World Health Organization (WHO) drinking water quality standards, the WHO recommends a maximum permissible limit of water pH from 6.5 to 8.5. This maximum permissible limit of water pH is considered as a safe range for water to be used for domestic use (World Health Organization, 2017). The same standards are adopted by KEBS for drinking. Although WHO does not give guideline for carbonated water, KEBS guideline as well as 2009/54/EC recommended a minimum pH of 4. Some of the points in the sampled area had water with pH values of less than 4, while none of the sampled sites had pH of more than 6.8 (Table 4.6). The high acidity of the water could be attributed to which carboxylic acid in the water due to the high CO₂ levels.

Temperature is a key parameter because it influences water chemistry. Palatability, viscosity, solubility, odours, and chemical reactions of water are influenced by temperature. There is no specified guideline for temperature in water used for drinking and domestic purposes. However, relatively high temperature impacts on the quality of water in a negative way by promoting micro-organisms, which interferes with taste, odor and color.. Therefore, it is vital for groundwater temperature not to be too high to not have microbial proliferation (Omer, 2019). The temperature for these waters ranged from 15.7 ± 0.05 °C to 16.6 ± 0.18 °C during the rainy season and 19 ± 0.18 °C to 20.7 ± 0.17 °C during the dry season (Table 4.6)

Turbidity can be defined as the clarity of a liquid. Others define it as the cloudiness or haziness of water. It is a key indicator of water quality and plays a great role in determining the cleanness of water (Mann*, Tam, Higgins and Rodrigues, 2007). During the rainy season, the highest turbidity was 320 ± 5.40 NTU at P4, and the lowest was 4.7 ± 0.16 NTU at P6. Similarly, during the dry season the highest turbidity was recorded at sampling site P2 – 261 ± 4.50 NTU, and the lowest was 3.2 NTU at P3 (Table 4.6). Most of the sampled points recorded higher turbidity values above the WHO and KEBS guidelines. The three bubbling points were recorded as high as 224 ± 5.25 and 320 ± 5.40 NTU in P4, 117 ± 1.25 , and 130 ± 3.15 NTU in P1, 261 ± 4.50 and 290 ± 4.70 NTU P2 during the dry and rainy season. Sampling P5 (sample collected from the river) also recorded values of 39.6 ± 1.20 and 50 ± 2.37 NTU during the dry and rainy seasons, respectively, above the WHO and the KEBS guidelines. Samples were drawn from the hand-dug well P3, and those from the spring P6 both conformed to the WHO and KEBS standards of <5 NTU (Table 4.6). The turbidity values were higher during the rainy

season than during the dry season; due to the transportation of suspended matter into the sampling point by the rain runoff water. Mbui et al., 2016 in their study of on the quality of water in Nairobi River found that turbidity was high in the rainy season compared to the dry season. The high turbidity on the river could be attributed to the fact that the river swept suspended particles as it flew downstream. The high turbidity values were recorded in the bubbling points P1, P2, and P4 as the bubbling process dislodging soil particles and given the fact that these points were located in a swampy area with a lot of suspended matter.

Water hardness is a water characteristic that indicative of total concentration of magnesium and calcium but in terms of calcium carbonate equivalent. Water total hardness may be as a result of pollution by industries or as result of natural geological processes (Christine et al., 2017). Water from sampling sites P1, P2, P3, P4 and P6 were all below 60 mg/L (Table 4.6) and can be classified as soft water, while the water sampled from the river, sampling site P5 would be classified as moderately hard water based on WHO classification (concentration below 60 mg/L as soft, 60-120 mg/L as moderately hard and 120-180 mg/L as very hard water).

Electrical conductivity basically looks at the ability of water to conduct electric current. It shows the percentage of charged ions in water. The electrical conductivity ranged from $55 \pm 0.8 \mu\text{S}/\text{cm}$ at P6 to $139 \pm 1.8 \mu\text{S}/\text{cm}$ at P5 in the wet season and $51 \pm 3.1 \mu\text{S}/\text{cm}$ in P6 to $156 \pm 4.2 \mu\text{S}/\text{cm}$ during the rainy season (Table 4.6). The high electrical conductivity in the river due to the high amounts of dissolved ions in the river water compared to the ground water. River dissolve much more minerals as it flows downstream compared to ground water. Moreover, the electrical conductivity (EC) of water as observed by Omer

2019 always has a directly correlation with the amount of total dissolved solid in the water. This was as well observed in this study, is also impacted by the water temperature level, whereby the warmer the water, the higher the water conductivity would be (Omer, 2019). That among other factors explains why the values recorded in the dry season were relative higher when then temperatures were also relatively higher. All the values reported in this study conformed to the WHO and KEBS electrical conductivity maximum of 400 $\mu\text{S}/\text{cm}$ permitted in drinking water (Meride & Ayenew, 2016).

Total dissolved solids (TDS) are inorganic salts as well as little amount of organic matter found in water in solution form. They include but not limited to potassium, calcium, sodium, magnesium cations as well as hydrogen carbonate, carbonates nitrates and chloride ions. In essence these ions can conduct electric current and that explains the direct relationship between TDS and electrical conductivity (Otieno, Kitur and Gathuru, 2017). The TDS for the sampled sites ranged from 24 ± 0.5 mg/L to 89 ± 2.1 mg/L as shown in table 4.6. These concentrations were within the KEBS maximum permissible values of 1200 mg/L for drinking water.

5.5 Statistical Analysis

The statistical analysis for the collected data was done using Analysis of Variance - one way ANOVA. According to Laerd Statistics One Way or single factor ANOVA is used to test whether there are any statistical significant differences between means of two or more independent groups. One way ANOVA was carried to check the significant differences between the mean of sample parameters in different seasons as well as between different sampling sites. There were significant differences between the means

in the different sampling site while the results showed no significant difference between seasons. This was true for all the parameters under consideration.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the general objective it is clear that the water cannot be used natural carbonated mineral water in its present state, as it does conform to KEBS, WHO and 2009/54/EC requirements in respect faecal and total coliform contamination in all the sampled sites, high turbidity in all sampled site except sample sites p3 and P6, due to low acidic of less than 4 pH value in in sampling sites P1 and P2.

All the anions determined in this study; nitrate, phosphates, sulphate and chloride were within the permissible limits recommended by KEBS and WHO for drinking water. Sodium and potassium was also within the range recommended by KEBS and WHO for drinking water. However, Kiplolok water contained higher amount of iron than those recommended by KEBS and WHO in sampling sites P1, P2, P4 and P5, as well manganese concentrations were higher in P1, P2, P3, and P4, than those recommended by KEBS and WHO. However, the higher concentration of iron in the water means that the water can be used as iron rich mineral water.

All the physico-chemical properties of the water were within the recommended range except that the water was found to be highly turbidity in sampling sites P1, P2, P4 and P5.

Water sampled from all the sampling sites except P% contained sufficient amount of dissolved CO₂ of above 1 g/L recommended by KEBS and 2009/54/EC for use as

carbonated water. On the other hand sampling sites P1 and P2 were highly acidic and not fit for use as carbonated water.

All the sampled sites were contaminated with faecal and total coliforms, against the KEBS and WHO recommendation that faecal and total coliform should not be present in drinking water.

The researched generally showed that there was significant difference between different sampled sites but there was no significance difference between same sampled sites in different seasons as per the single factor ANOVA performed on the obtained data.

6.2 Recommendations

The area where these bubbling springs are located should be fenced off. This will help preserve the spring and minimize microbial contamination of the waters

Water treatment using such methods like coagulation flocculation to reduce the turbidity and chlorination to eliminate bacteria contamination are recommended to ensure that the water is safe for human consumption.

The water pH should be adjusted to the minimum of 4, through addition of ionized water, to conform to the 2009/54/EC and KEBS standards for natural carbonated water.

After the treatment of the water to reduce turbidity and eliminate microbial contamination, kiplolok water being rich in iron could be recommended as iron supplement water.

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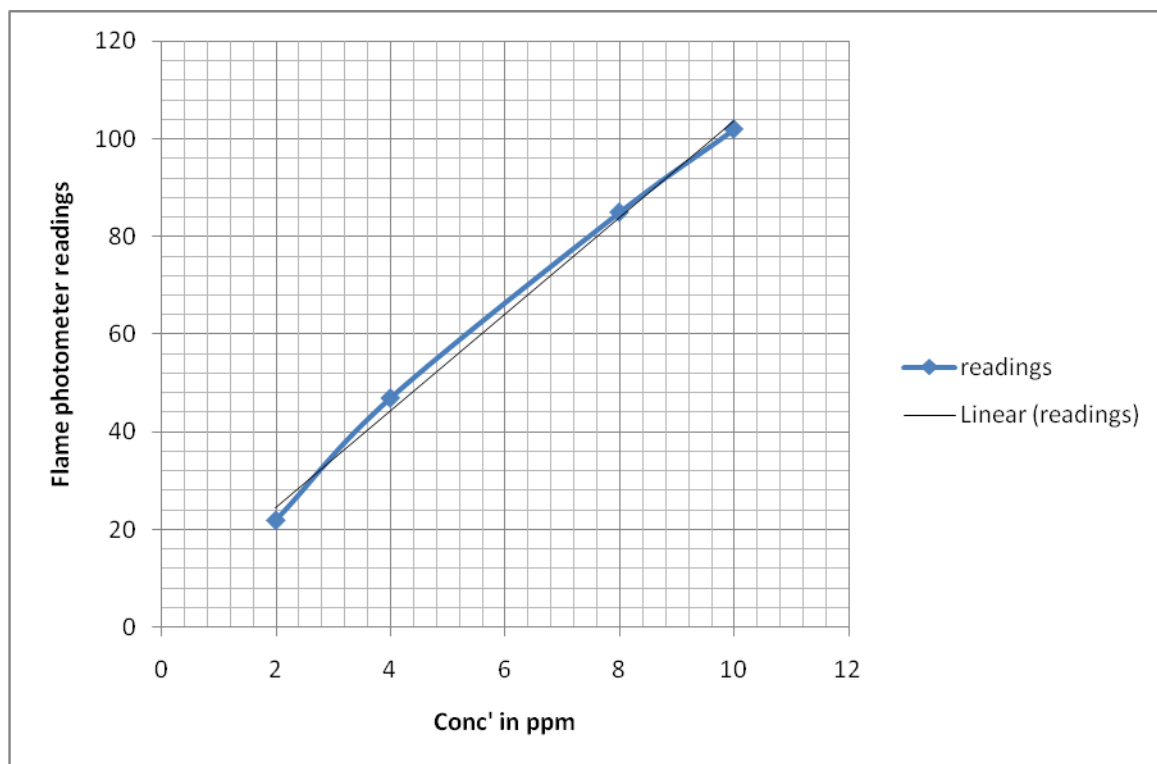
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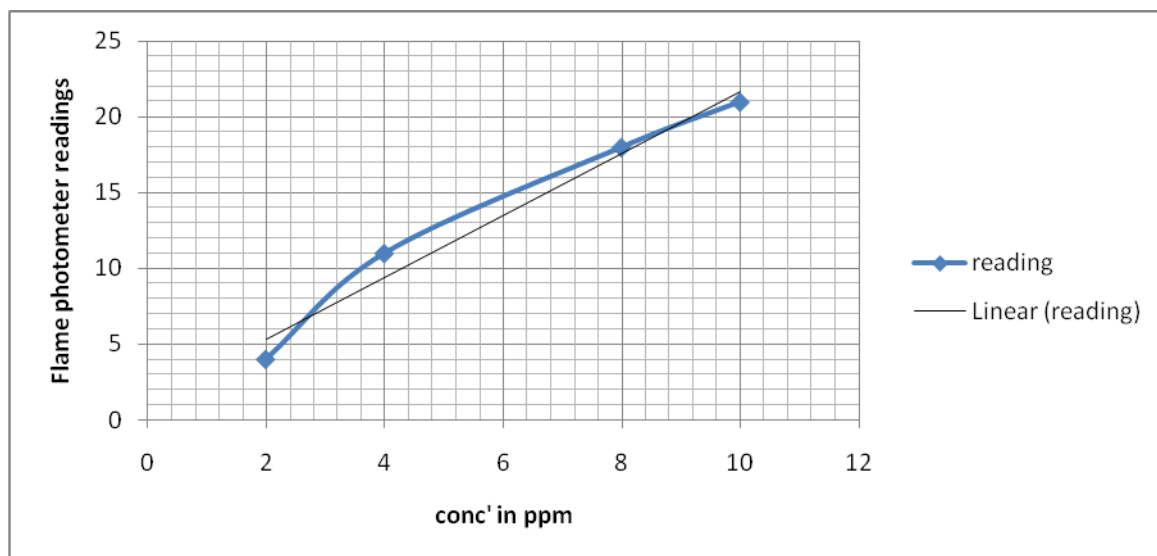
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APPENDICES

Appendix I: Potassium Standards calibration curve



Appendix II: Sodium Standards calibration curve

Appendix III a: Iron ANOVA Between seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.264033	1	0.264033	0.112514	0.744231	4.964603
Within Groups	23.46673	10	2.346673			
Total	23.73077	11				

Appendix III b: Iron ANOVA between sampling points

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	23.25097	5	4.650193	58.15165	5.3E-05	4.387374
Within Groups	0.4798	6	0.079967			
Total	23.73077	11				

Appendix IV a: Manganese ANOVA between seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.003333	1	0.003333	0.033557	0.858313	4.964603
Within Groups	0.993333	10	0.099333			
Total	0.996667	11				

Appendix IV b: Manganese ANOVA between sampling points

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.906667	5	0.181333	12.08889	0.00435	4.387374
Within Groups	0.09	6	0.015			
Total	0.996667	11				

Appendix V a: Nitrate ANOVA between seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.000833	1	0.000833	0.000192	0.989205	4.964603
Within Groups	43.30833	10	4.330833			
Total	43.30917	11				

Appendix V b: Nitrate ANOVA between sampling points

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	42.11417	5	8.422833	42.29038	0.000134	4.387374
Within Groups	1.195	6	0.199167			
Total	43.30917	11				

Appendix VI a: Phosphate ANOVA between seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.0507	1	0.0507	0.907301	0.363287	4.964603
Within Groups	0.5588	10	0.05588			
Total	0.6095	11				

Appendix VI b: Phosphate ANOVA between sampling points

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.4472	5	0.08944	3.30647	0.088847	4.387374
Within Groups	0.1623	6	0.02705			
Total	0.6095	11				

Appendix VII a: Sulfate ANOVA between seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.083333	1	0.083333	0.172414	0.686744	4.964603
Within Groups	4.833333	10	0.483333			
Total	4.916667	11				

Appendix VII b: Sulfate ANOVA between sampling points

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	3.416667	5	0.683333	2.733333	0.126478	4.387374
Within Groups	1.5	6	0.25			
Total	4.916667	11				

Appendix VIII a: Chloride ANOVA between Seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.181056	1	0.181056	0.037411	0.850503	4.964603
Within Groups	48.39666	10	4.839666			
Total	48.57772	11				

Appendix VIII b: Sulfate ANOVA between sampling points

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	46.50646	5	9.301291	26.94381	0.000485	4.387374
Within Groups	2.071264	6	0.345211			
Total	48.57772	11				

Appendix IX a: pH ANOVA between seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	0.261075	1	0.261075	0.207916	0.658148	4.964603
Within Groups	12.55675	10	1.255675			
Total	12.81783	11				

Appendix IX b: pH ANOVA between sampling points

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between						
Groups	12.20548	5	2.441095	23.91862	0.000677	4.387374
Within Groups	0.61235	6	0.102058			
Total	12.81783	11				

Appendix X a: Dissolved CO₂ ANOVA between seasons

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	0.2883	1	0.2883	0.35804	0.562903	4.964603
Within Groups	8.052167	10	0.805217			
Total	8.340467	11				

Appendix X b: Dissolved CO₂ ANOVA between sampling points

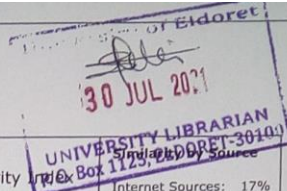
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	7.708967	5	1.541793	14.64887	0.00261	4.387374
Within Groups	0.6315	6	0.10525			
Total	8.340467	11				

Appendix XI: Similarity Report

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