RELATIONSHIP BETWEEN NITRATES, PHOSPHATES IN WATER, IODINE LEVELS IN TABLE SALT AND GOITER OCCURENCE: A CASE STUDY OF NANDI HILLS, KENYA

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DECLARATION AND APPROVAL

Declaration by the Student

I declare that this thesis is my original work and has not been presented for examination in any academic institution. No part of this work may be reproduced without prior permission of the author and/or University of Eldoret.

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DEDICATION

This research project is dedicated to my parents for laying down the foundation of my education and for their moral support. I also dedicate this work to my brothers and sisters for their encouragement and motivation.

ABSTRACT

Nitrates and phosphates are among the major inorganic pollutants in the environment, primarily contributed by nitrogenous and phosphate fertilizers, organic manures, human and animal wastes, and industrial effluents through biochemical activities of microorganisms. Beside other known detrimental effects, research findings suggest that increased nitrate and/or phosphate intake affects the functioning of the thyroid gland in humans. In Kenya, goiter remains endemic despite iodization of table salt in households. Notably, the area around Nandi hills in Nandi County has recorded high incidences of goiter in the recent past. This indicates that factors such as inorganic ion content of water (among others) may have etiological role in endemic goiter cases in the region. This study sought to determine the etiological factors leading to observed high prevalence of goiter. This was achieved through determination of concentration of nitrate ions and phosphate ions in drinking water and urine. Three samples were collected; urine, table salt and drinking water (stream and borehole). Urine samples were analyzed to determine the concentration of nitrate ions, phosphate ions, and Iodate ions using cadmium reduction method, UV-visible spectrophotometry and Salifert Iodine pro test kits respectively. Iodate ions in table salt were quantified using the same kits. From the results obtained in the study, stream water recorded nitrate and phosphate concentrations of 2.91±0.74 and 0.30±0.08 mg/l respectively. In addition, there was a significant difference ($P<0.05$) in both nitrate and phosphate levels among the three sampling points in the three streams. However, there was no significant difference ($P>0.05$) in both nitrate and phosphate concentrations among the three streams used in the study. In borehole water, nitrate and phosphate levels were 1.69±0.79 and 0.29±0.07mg/l respectively. Both nitrate and phosphate levels in stream and borehole water were found to be within the WHO acceptable limits. On the other hand, nitrate and phosphate levels in urine samples were 0.53±0.30 and 709.59±5.57mg/l respectively. Iodine concentration in salt used by respondents in the study area ranged between 43.78±9.47 and 69.21±5.08 when titration and saltPADs methods were used respectively. Pearson Correlation results reported an insignificant positive association ($P>0.05$; $r>0$)between nitrate, phosphate and iodine ions and occurrence of goiter in the study. The study therefore concluded that concentrations of nitrate and phosphate ions in drinking water and iodine ions in table salt are not the main cause of goiter in the study area. This calls for additional studies to be carried out on other possible causes of goiter in the study area.

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LIST OF ACRONYMS

ANOVA	Analysis of variance
CAN	Calcium Ammonium Nitrate
DAP	Diammonium Phosphate
HCG	Human Chorionic Gonadotropin
IDD	Iodine Deficiency Disorders
MTRH	Moi Teaching and Referral Hospital
NACOSTI	National Council of Science, Technology and Innovation
NIS	Sodium-Iodide Symporter
PTH	Parathyroid Hormone
TGP	Total Goiter Prevalence
TSH	Thyroid Stimulating Hormone
TSP	Triple Super Phosphate
VOC	Volatile Organic Compounds
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Ground and surface water has been utilized for drinking purposes for a long time. Surface water sources include streams, rivers, marshes, lagoons, ponds, and lakes while ground water sources include mainly boreholes (Chapra, 2008). The current progression of human civilization has put serious concerns to the safety of ground and surface water for drinking purposes. This is mainly because of the current rate of water pollution. This form of environmental degradation occurs when pollutants are directly or indirectly discharged into surface and/or ground water bodies without adequate treatment to remove harmful compounds (Hammer& Chadik, 2009). Water pollution is a major global problem mainly because of its serious effects on the entire biosphere (Pimentel *et al*.,2004). The specific contaminants leading to pollution of water include a wide spectrum of chemicals, pathogens, and/or physical changes such as elevated temperature and discoloration (Schawarzenbach *et al*., 2010). While many of the chemicals and substances that are regulated may be naturally occurring (calcium, sodium, iron, manganese, etc.) the concentration is often the key in determining what is a natural component of water and what is a contaminant. High concentrations of naturally occurring substances can have negative impacts on aquatic flora and fauna (WHO, 2004).

Currently, the high water pollution load comes from chemicals which include detergents, disinfection by-products, food processing waste, insecticides, herbicides, petroleum, hydrocarbons, volatile organic compounds (VOCs), chlorinated solvents, perchlorate, pharmaceutical drugs and their metabolites, ammonia from food processing waste, chemical waste such as industrial by-products, fertilizers, heavy metals and silt (WHO, 2004; Hammmer & Chadik, 2009; Forstnet & Wittmann, 2012).

Nitrates (NO_3^-) and phosphates (PO_4^{3-}) are among the major inorganic pollutants contributed by nitrogenous and phosphate fertilizers, organic manures, human and animal wastes and industrial effluents through the biochemical activities of microorganisms (Almasri & Kahuarachi, 2004; Goel, 2006).

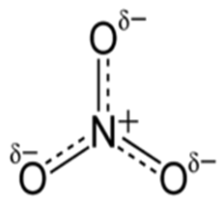

Figure 1.1: Nitrate ion (Goel, 2006)

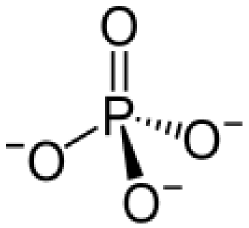

Figure 1. 2: Phosphate ion (Goel, 2006)

Excessive use of nitrogenous fertilizers such as Calcium Ammonium Nitrate (CAN) in agriculture has been one of the primary sources of high nitrate ions in water sources. Apart from nitrate ions, nitrogen is applied in ammonium (NH_4^+) and amide (NH_2^-) forms, which generates nitrate in soil system through mineralization, which is fairly rapid in tropical and subtropical soils. Livestock feeding, barnyards, septic tanks, animal and human contamination are the other important sources contributing high amounts of NO_3^- (Goel, 2006; Savci, 2012). Due to its high solubility in water and low retention by soil particles, nitrate ions are prone to leaching to the subsoil layers and ultimately to the groundwater, if not taken up by plants or denitrified to N_2O and N_2 (Majumdart & Gupta, 2000). The rate of leaching is governed by the soil properties and amount of water present in the soil system. The arrival of nitrate to ground and surface water can be enhanced by shallow groundwater table; excessive application of nitrogenous fertilizers, manures and irrigation; and abundant rainfall. Once ingested, nitrates are readily absorbed from the proximal small intestine and rapidly equilibrate with body fluids. In rats, about 50% of an oral dose was detected in the carcass within 1 hour; in humans, peak levels were achieved in serum, saliva and urine within 1 to 3 hours (Hungate, 2013). There is little absorption from the stomach in most species, although some has been reported from the rumen of cattle (Hungate, 2013).

Excessive use of phosphate fertilizers in agriculture has been known to be one of the major sources of high phosphates in water sources. In addition, waste-waters from laundering agents contain phosphates; Most laundry detergents contain approximately 35% to 75% sodium triphosphate ($Na_5P_3O_{10}$) (Bajpai & Tyagi, 2007). Phosphates exist in three forms: orthophosphate, metaphosphate (or polyphosphate) and organically bound phosphate with each compound bearing phosphorous in a different chemical arrangement (Georganta & Grigoropoulou, 2007). Orthophosphates applied to agricultural or residential lands as fertilizers are carried into the surface water. In addition, leaching events can cause the vertical migration of the phosphates into the groundwater system causing pollution. Different organizations and countries have set standards for nitrates and phosphates in drinking water, to safeguard public health from the hazards associated with high concentrations of the chemicals.

The historic area of concern with respect to nitrate exposure and human health is methemoglobinemia, or blue baby syndrome. Blue baby syndrome occurs when nitrite mediates the oxidation of the heme ion in hemoglobin (an oxygen-carrying protein pigment in red blood cells) to form methemoglobin (Greer & Shannon, 2005). This can result in anemic hypoxia (oxygen-deficient blood), which can be life-threatening for an infant (Fewtrell, 2004).

Lately, it has been also suggested that increased nitrate and/or phosphate intake affects the function of the thyroid gland, as was observed in a study in pigs by competitive inhibition of iodide transport leading to decreased thyroid hormone secretion, followed by an increase in thyroid-stimulating hormone (Zoeller, 2007; Eskiocak *et al*., 2005). This has been theorized to happen through the inhibition of steroidogenesis without involving the guanylatecyclase-cyclic guanosine monophosphate pathway (Zaki *et al*., 2004). This has been hypothesized to be a major cause of goiter in humans though no conclusive studies have been conducted.

Goiter occurs with varying intensity in almost every country though some few countries appear to be entirely free from it. It is characterized by a swelling of the larynx resulting from enlargement of the thyroid gland (thyromegaly) normally associated with a thyroid gland that is not functioning properly (Knudsen, 2000). Goiter is caused by deficiency of iodine in the human body. Globally, the Total Goiter Prevalence (TGP) in the general population is estimated to be 15.8%, varying between 4.7% in America to 28.3% in Africa (Andersso*n et al*., 2005). Worldwide, over 90.54% (approximately 200 million people) cases of goiter are caused by iodine deficiency (De Benoist *et al*., 2004). Selenium deficiency is also considered a contributing factor (Aydin *et al*., 2002). In countries that use iodized salt, Hashimoto's thyroiditis is the most common cause. Goiter can also result from cyanide poisoning; this is particularly common in tropical countries where people eat the cyanide-rich cassava root as the staple food (Sousa *et al*., 2002)

1.2 Statement of the Problem

High incidences of goiter in the world have been reported in high mountain region (in Alpine valleys, in the Pyrenees, on the slopes of the Himalayas, and along the Cordillera of the Andes) though goiter is also known to occur within low-lying areas and even at sea level.

In Kenya, Goiter remains endemic despite iodization of salt. Notably, the area around Nandi Hills in Nandi County has recorded high incidences of goiter in the recent past. According to the Kapsabet District Hospital and Moi Teaching and Referral Hospital (MTRH) records, goiter accounts for 50 - 72% of the diseases in the Nandi Hills area (Ali *et al.,* 2014). This is despite the fact that the table salt used in most of the households in Kenya is iodized. This suggests that other etiological factors such as inorganic content of water may have a role in the causation of endemic goiter cases in the region. In addition, tea farming is the main economic activity in the study area. In Nandi Hills tea plantations, about 5,000 hectares of land are under mature tea and fertilizer is applied at a rate of 140 Kg /ha/year, with N: P: K being the commonly used fertilizer (EPK, 2006). Therefore, these inorganic ions can be discharged to water bodies through leaching and surface run-off. This study thus aims at determining the etiological factors that potentially cause the high prevalence of goiter in this region. This will be achieved through the determination of the concentration of nitrates and phosphates in the drinking water commonly used in this area and urine samples. Additionally, the research will quantify the level of iodine in urine and salt samples.

1.3 Objectives of the Study

1.3.1 General Objective

To determine the concentration of nitrates and phosphates in drinking water and urine samples from inhabitants of Nandi Hills.

1.3.2 Specific Objectives

1. To determine the concentration of nitrates and phosphates in drinking water samples collected from streams and boreholes in Nandi Hills.
2. To determine the concentration of nitrates and phosphates in urine samples from selected inhabitants of Nandi Hills.
3. To establish iodine concentration in table salt from the inhabitants of Nandi hills
4. To determine the relationship between the concentration of nitrates ions, phosphates ions and iodine ions in the samples and the prevalence of goiter in the study area.

1.4 Justification

Several studies have shown that the endemic goiter in many countries including Kenya is associated with iodine deficiency (Lamberg, 1993: Boyages, 1993; Bayram *et al.,* 2003). However, some studies have reported that high exposure to inorganic ions like nitrate and phosphate has an effect on goiter development (Zoeller, 2007). Studies by WHO on prevalence of goiter among Kenyan populace by the year 2013 indicated a reducing trend as compared to other prevoius years. However, in Nandi Hills region, the prevalence seems to be increasing. This is despite the fact that the table salt used in most of the households in Nandi Hills is iodized. The thyroid gland plays a major role in up-taking iodide (I^-) and synthesizing new thyroid hormones. Nevertheless, presence of inorganic ions like nitrate inhibits thyroid function by blocking the sodium-iodide symporter (NIS) (Sherrill *et al.*, 2004). Therefore, there is need to determine the concentrations of inorganic ions in drinking water in Nandi Hills so as to determine the possible causes of goiter in this region.

1.5 Significance of the Study

The findings from this study are expected to bring into the limelight potential contributory factors leading to the increased prevalence of goiter in the Nandi Hills region. Additionally, the findings will give information whether high nitrates and phosphates in drinking water can be associated with increased incidence of goiter. The study findings will also serve as a guide to health practitioners and policy makers in their efforts to combat the rising trend of goiter in Nandi Hills region.

1.6 Scope of the Study

There are a number of possible sources of man-made water contaminants. However, this study will be biased only on nitrates and phosphates as contaminants of drinking water. This is mainly because only nitrates and phosphates are theorized to be the inorganic contaminants of water which may have an etiological role in the causation of endemic goiter cases.

The study will also be purposively conducted in Nandi Hills. This is because Nandi Hills is the only area which has recorded noticeable increase of goiter cases despite the overall decrease of the prevalence of the disease in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Goiter

Goiter is a disease characterized by an abnormal swelling of the thyroid gland that causes a lump to form in the neck (Tonacchera *et al.,* 2010). The thyroid gland is a small butterfly-shaped gland in the neck, just in front of the windpipe (trachea). It produces thyroid hormones, which help regulate the body's metabolism (Fagman & Nilsson, 2010). The thyroid gland is not usually noticeable, but if it swells, it produces a lump on the neck known as goiter. The size of goiter can vary from person to person. In most cases, the swelling is small and does not cause any symptoms. However, in more severe cases, the swelling can increase significantly and affect breathing and swallowing (Barrett *et al.,* 2010).

2.1.1 Causes of Goiter

There are several known causes of goiter. The main one is iodine deficiency. Iodine is found primarily in sea water (sea foods) and also in common table salt. Iodine is an essential trace element (Mertz, 2012). It is a major component of the thyroid hormones thyroxine and triiodothyronine (Pitt-Rivers& Tata, 2013). In areas where there is little iodine in the diet typically remote inland areas where no marine foods are eaten, iodine deficiency is common. It is also common in mountainous regions of the world where food is grown in iodine-poor soil (Li& Eastman, 2012). Although a lack of dietary iodine is the main cause of goiter in many parts of the world, this is not often the case in countries where iodine is routinely added to table salt and other foods.

Another known cause of goiter is Graves' disease. Graves' disease is an immune system disorder that results in the overproduction of thyroid hormones, a condition known as hyperthyroidism (Hemminki *et al.,* 2010). Although a number of disorders may result in hyperthyroidism, Graves' disease is a common cause. In Graves' disease, antibodies produced by the immune system mistakenly attack the thyroid gland, causing it to produce excess thyroxine. This overstimulation causes the thyroid to swell resulting to goiter (Menconi *et al.,* 2014).

Hashimoto's disease is another known cause of goiter. Hashimoto's disease is a condition in which the immune system attacks the thyroid (Umar*et al.,* 2010). Like Graves' disease, Hashimoto's disease is an autoimmune disorder, but instead of causing the thyroid to produce too much hormone, Hashimoto's damages the thyroid so that it produces too little (Boelaert *et al.,* 2010). In response to low hormone level, the pituitary gland produces more thyroid stimulating hormone to stimulate the thyroid, which then causes the gland to enlarge causing goiter.

Multinodular goiter is another known cause for goiter. In this condition, several solid or fluid-filled lumps called nodules develop in both sides of the thy roid, resulting in overall enlargement of the gland (Bahn& Castro, 2011). Solitary thyroid nodules can also cause goiter. In this case, a single nodule develops in one part of the thyroid gland (Paschke *et al.*, 2011). Most nodules are noncancerous (benign) and do not lead to cancer.

Goiter can also result in patients with thyroid cancer. Thyroid cancer is far less common than benign thyroid nodules. Cancer of the thyroid often appears as an enlargement on one side of the thyroid (Pazaitou-Panayiotou *et al.,* 2012).

In pregnancy, a hormone called human chorionic gonadotropin (HCG), may cause the thyroid gland to enlarge slightly resulting to goiter. Human placenta synthesizes glycoprotein, steroids, and protein hormones during pregnancy (Harrington & Campbells, 1993). The production of HCG by placenta in the first trimester of pregnancy is critical for implantation and maintenance of the blastocyst (Petraglia *et al.,* 1990). HCG can stimulate the thyroid gland during first trimester of pregnancy because of its structural resemblance with TSH (Fantz *et al.,* 1999). During pregnancy, thyroid hormones have an important role in embryogenesis and foetal brain development (Haddow *et al.,* 1999). Therefore, thyroid function is frequently assessed during pregnancy, both to evaluate suspected thyroid abnormalities, and to monitor the status of preexisting thyroid disease (Zarghami *et al.*, 2005).

Finally, Thyroiditis can also cause goiter. This is an inflammatory condition that can cause pain and swelling in the thyroid (Jankovic *et al.,* 2013). It may also cause an over- or underproduction of thyroxine.

2.1.2 Prevalence of Goiter

Goiter cases are minimal in affluent countries where table salt is supplemented with iodine, however, it is still prevalent in most countries as shown in Table 2.1 below. Globally, the prevalence of goiter is estimated to be 12% (Li& Eastman, 2012). The highest regional goiter prevalence is in the Eastern Mediterranean Region (22.9 %), while over 42.5 % of the population is considered to be at-risk of iodine deficiency in that Region (Andersson*et al.,* 2012). In absolute terms, Southeast Asia including India, Bangladesh and Indonesia and the Western Pacific together account for more than 50% of the world's total population at-risk of iodine deficiency disorders (IDDs) (Andersson*et al.,* 2010). There is still a non-negligible IDDs problem in most countries in Europe (even Western Europe, including 10 million people at-risk in

Germany), necessitating continuing control measures and vigilance (Zimmermann, 2013). The largest increases in populations-at-risk are in the American, Eastern Mediterranean, European and Southeast Asian Regions (Andersson *et al.,* 2012).

In Africa, goiter is endemic in many countries notably Congo, Uganda, Kenya and Sudan. The prevalence is as high as 81% in some parts of these countries (Elnour *et al*., 2000). It is the chief consequence of iodine deficiency, resulting from either low iodine intake or ingestion of goitrogens (Narwal, 2013). Iodine deficiency also occurs in lowland regions far from the oceans, such as central Africa (Zimmermann & Andersson, 2012). The extreme form of iodine deficiency, endemic cretinism has been well characterized in Central Africa, where up to 2–6% of the overall population may be affected (Zimmermann *et al.,* 2003). Gross neurological defects are also seen in African populations and were described in 10% of patients with cretinism in the Kivu area of Democratic Republic of Congo (Zimmermann *et al.,* 2003).

A national micronutrient survey conducted in 45 districts in Kenya found total goiter rates as 16.3% (FAO, 2005). Three had no goiter problems, while 30 had mild iodine deficiency disorders (IDD) with goiter prevalence between 5 and 19%, 7 had moderate problem (TGR 20-29%) and 5 districts were noted to have goiter prevalence's greater than 30%. Survey data from Rift Valley where Nandi Hills is located indicated total goiter prevalence as 20% (FAO, 2005). Cases of goiter have been reported in Nandi Hills despite the use of iodized salt, raising a public health concern (Adwok, 2006). Furthermore, doctors who carried out goiter surgical operation services in three towns of the north Rift region reported that goiter disease is highly prevalent (Wamalwa, 2012).

Table 2.1: Total Number of People and Percent of Regional Population Living in Areas At-Risk of Iodine Deficiency Disorders (TGR> 5%) and Affected by Goiter

WHO Region	**Population (millions)**	**Population At-Risk (millions)**	**% of Region**	**% of Global Total**	**Population Affected by Goiter (millions)**	**% of Region**	**% of Global Total**
Africa	550	181	32.8	11.5	86	15.6	13.1
Americas	727	168	23.1	10.7	63	8.7	9.6
Eastern Mediterranean	406	173	42.6	11.0	93	22.9	14.2
Europe	847	141	16.7	9.0	97	11.4-	14.8
Southeast Asia	1355	486	35-9	30.9	176	13.0	26.8
Western Pacific	1353	423	27.2	26.9	141	9.0	21.5
Total	5,438	1,572	28.9	100.0	655	12.0	100

Source: WHO (2010)

2.1.3 Treatment of Goiter

The well-known treatment for goiter is via medications**.** In the case of hypothyroidism, thyroid hormone replacement with levothyroxine (Levothroid, Synthroid) usually resolves the symptoms of hypothyroidism as well as slowing the release of thyroid-stimulating hormone from the pituitary gland, often decreasing the size of the goiter (Hughes & Eastman, 2012). For inflammation of the thyroid gland, medical practitioners usually prescribe aspirin or a corticosteroid medication to treat the inflammation (Tonacchera *et al.,* 2010). Goiters associated with hyperthyroidism require medications to normalize hormone levels.

A less common remedy to goiter is surgery**.** This involves the removal of all or part of the thyroid gland (total or partial thyroidectomy) especially if the patient has a large goiter that is uncomfortable or causes difficulty breathing or swallowing, or in some cases, if a nodular goiter is causing hyperthyroidism (Hughes & Eastman, 2012). Surgery is also the treatment for thyroid cancer (Memon *et al.*, 2010).

In some cases, radioactive iodine is used to treat an overactive thyroid gland. The radioactive iodine is taken orally and reaches the thyroid gland through the bloodstream, destroying thyroid cells (Haymart *et al.*, 2011). The treatment results in diminished size of the goiter, but eventually may also cause an underactive thyroid gland.

2.2 Nitrates

Nitrate is a polyatomic ion with the molecular formula NO_3^- and a molecular mass of 62.0049 g/mol. Nitrates also describe the organic functional group $RONO_2$ (Beckey, 2013). Nitrate salts are found naturally on earth as large deposits, particularly of Nitratine. Nitrates are mainly produced for use as fertilizers in agriculture because of their high solubility and biodegradability (Sebilo *et al.*, 2013). The main nitrates are ammonium, sodium, potassium, and calcium salts. Almost all inorganic nitrate salts are soluble in water at standard temperature and pressure (Armarego & Chai, 2013). A rich source of inorganic nitrate in the human body comes from diets rich in leafy green foods, such as spinach and arugula.

2.2.1 Nitrates Pollution

Currently, Nitrate is a key problem as a contaminant of drinking water (primarily from groundwater and wells) due to its harmful biological effects (Burow *et al.*, 2010). According to Stevens (2012), agricultural activities, primarily crop and livestock production, account for over 80% of all nitrogen added to the environment. Fertilizers form the single largest source of nitrates in the world and not surprisingly, agricultural areas have the highest rates of nitrate contaminated water (Conway& Pretty, 2013). Animal manure is the second largest source of nitrates in the environment, accounting for 13 billion pounds per year (Kuzelka & Ennenga, 2013).

Non-agricultural sources of nitrogen contribute less than 20% of the nitrogen released into the environment (Fowler *et al.,* 2013). 6% is released from point sources (basically pipes) into water bodies, while 14% is deposited from atmospheric sources. Point sources in urban watersheds can cause significant localized nitrate problems in surface waters or individual wells. According to Loehr (2012), a variety of point sources contribute approximately 2.6 billion pounds of nitrates to the environment into surface waters each year. Municipal sewage plants account for 80% of point source nitrates discharges; individual septic tanks and a number of industrial sources account for the rest (Wang *et al.,* 2012).

Nitrates are also deposited in soil and water from the atmosphere, where it enters from an array of sources as nitrogen oxide emissions from coal or oil burning electric utilities or other industries or from automobiles, trucks or buses (Berner & Berner, 2012).

2.2.2 Effects of Nitrates Pollution

High concentrations of nitrates in the body can cause methemoglobinemia, and have been cited as a risk factor in developing gastric and intestinal cancer (Tang*et al.,* 2011). Methemoglobinemia most often affects infants of less than six months of age. Methemoglobinemia is the condition in the blood which causes infant cyanosis, or blue-baby syndrome (Trapp& Will, 2010). Methemoglobin is probably formed in the intestinal tract of an infant when a bacterium converts the nitrate ion to nitrite ion (Ashurst & Wasson, 2011). One nitrite molecule then reacts with two molecules of hemoglobin to form methemoglobin. This altered form of blood protein prevents the blood cells from absorbing oxygen which leads to slow suffocation of the infant which may lead to death rapidly (Meisenberg & Simmons, 2011). Because of the oxygen deprivation, the infant will often take on a blue or purple tinge in the lips and extremities, hence the name, blue baby syndrome (Ashurst & Wasson, 2011). Other signs of infant methemoglobinemia are gastrointestinal disturbances, such as vomiting and diarrhea, relative absence of distress when severely cyanotic but irritable when mildly cyanotic and chocolate-brown colored blood (Tang *et al.*, 2011).

Nitrate pollution has also been associated with stomach and gastrointestinal cancer. Although many studies have been performed attempting to link stomach and gastrointestinal cancer to nitrate intake, there is no conclusive evidence for correlation. Researchers claim that nitrate represents such a potential risk because of nitrosation reactions which, with appropriate substrates present, form N-nitroso compounds which are strongly carcinogenic in animals (Bryan *et al.*, 2012).

Nitrate pollution also affects the environment negatively. The widely known environmental adverse effect of nitrate pollution is eutrophication. This is the process by which a body of water acquires a high concentration of nutrients, especially phosphates and nitrates. These typically promote excessive growth of algae. As the algae die and decompose, high levels of organic matter and the decomposing organisms deplete the water of available oxygen, causing the death of other organisms, such as fish (Smolders *et al.,* 2010).

Another environmental effect is anoxia. Anoxia is a lack of oxygen caused by excessive nutrients in waterways which triggers algae growth. When the plants die and decay, oxygen is stripped from the water, which then turns green or milky white and gives off a strong rotten egg odour. The lack of oxygen is often deadly for invertebrates, fish and shellfish (Jenkyns, 2010).

Due to these health risks, a great deal of emphasis has been placed on finding effective treatment processes to reduce nitrate concentrations to safe levels (Hwang *et al.,* 2010; Bhatnagar & Sillanpää, 2011). An even more important facet to reduce the problem is prevention measures to stop the leaching of nitrate from the soil. Some suggest that reducing the amount of fertilizers used in agriculture will help alleviate the problem, and may not hurt crop yields (Masclaux-Daubresse *et al.,* 2010). Other new developments in leach pits and slurry stores help to control the nitrate that comes from stored manure (Jadhao, 2013). By installing these prevention methods and reducing the amount of fertilizer used, the concentration of nitrate in the groundwater can be reduced over time. Treatment processes, such as ion exchange can have an immediate effect on reducing levels in drinking water. These processes do not remove the entire nitrate, but can help to bring the concentration down to the suggested level of 10mg/L (Chand *et al.,* 2011).

2.2.3 Putative Relationship between Nitrate Intake and the Endocrine System

It has been suggested that increased nitrate intake affects the function of the thyroid gland in humans by competitive inhibition of iodide transport leading to decreased thyroid hormone secretion, followed by an increase in thyroid-stimulating hormone. Research (Ward *et al.,* 2010; Kilfoy *et al.*, 2011; Aschebrook-Kilfoy *et al.,* 2013; Speijers & Van den Brandt, 2003; Pearce & Braverman, 2009), has shown that ingested nitrate inhibits thyroid uptake of iodide by binding to the sodium-iodide symporter on the surface of thyroid follicles. In turn the levels of the thyroid hormones triiodothyronine (T3) and thyroxin (T4), reduces significantly leading to an increase in thyroid stimulating hormone (TSH). TSH controls thyroid hormone production through a negative feedback loop. Chronic stimulation of the thyroid gland by TSH can lead to proliferative changes in follicular cells, including hypertrophy and hyperplasia as well as neoplasia. There is no evidence from human studies that exposure to elevated nitrate levels in drinking water is associated with increased thyroid volume and increased frequency of subclinical thyroid disorders. Nitrate and nitrite are also precursors in the endogenous formation of N-nitroso compounds, which are potent animal carcinogens that cause thyroid and many other tumors in animal models (Ward *et al.*, 2010).

2.3 Phosphates

A phosphate (PO_4^{3-}) as an inorganic chemical is a salt of phosphoric acid (Skoog *et al.*, 2013). In organic chemistry, a phosphate, or organophosphate, is an ester of phosphoric acid (Carey & Sundberg, 2007). Phosphates is the naturally occurring form of the element phosphorus, found in many phosphate minerals. In mineralogy and geology, phosphate refers to a rock or ore containing phosphate ions (Corbridge, 2013). Inorganic phosphates are mined to obtain phosphorus for use in agriculture and industry. Phosphates exist in three forms: orthophosphate, metaphosphate (or polyphosphate) and organically bound phosphate each compound contains phosphorous in a different chemical arrangement. These forms of phosphate occur in living and decaying plant and animal remains, as free ions or weakly chemically bound in aqueous systems, chemically bonded to sediments and soils, or as mineralized compounds in soil, rocks, and sediments (Durif, 2013).

2.3.1 Phosphates Pollution

Phosphates have caused increasing attention recently. There are several sources of phosphate pollution. Lawn and garden fertilizers especially TSP and DAP are often implicated as the major source of phosphate pollution. Wastewater, from laundering agents also contains phosphates, which are a water pollutant (Förstner & Wittmann, 2012). The non-point sources of phosphates include: natural decomposition of rocks and minerals, storm water runoff, agricultural runoff, erosion and sedimentation, atmospheric deposition, and direct input by animals/wildlife.

Once in the water bodies, phosphates stimulate the growth of plankton and aquatic plants which provide food for larger organisms, including zooplankton, fish, humans, and other mammals. This process leads to eutrophication. Eutrophication is the progressive over-fertilization of water, in which festering masses of algae's blooms, choking rivers and lakes. Phosphorus compounds act as a fertilizer for all plant life and are implicated in eutrophication (Smolders *et al.*, 2010). Many countries control phosphate levels and some such as Switzerland has banned the use of phosphates.

2.3.2 Putative Relationship between Phosphates Intake and the Endocrine System

There are variable reports linking high serum phosphorous levels to hyperthyroidism. Most of the studies indicate hyperphosphatemic state (Dhanwal, 2011). Hyperphosphatemia in hyperthyroidism has been explained on the basis of an enhanced tissue catabolism emanating from an excess input of phosphorous to the plasma pool from bone and tissues and lower fractional clearance of phosphorous and increased renal tubular re-absorption of phosphorous (Martin& González, 2011). The changes in serum phosphorous are due to suppressed Parathyroid hormone (PTH) levels as well as direct effects of thyroid hormones on tissue phosphate metabolism and renal phosphate handling. These effects lead to increased filtered load of phosphorous causing hyperthyroidism (Dhanwal, 2011).

2.4 Related Studies

Reviewed literature reveals that very few studies have been done linking goiter and intake of excessive nitrates and/or phosphates. Kilfoy *et al.* (2011) evaluated dietary nitrate and nitrite intake and thyroid cancer risk overall and for subtypes in a large prospective cohort of 490,194 men and women, ages 50–71 years. Their findings showed that among men, increasing nitrate intake was positively associated with thyroid cancer risk (relative risk [RR] for the highest quintile versus lowest quintile RR = 2.28, 95%). However, no trend was observed with intake among women (*p*-trend = 0.61). From their findings, they concluded that nitrite intake was not associated with risk of thyroid cancer for either men or women. However, an evaluation of risk for the two main types of thyroid cancer revealed a positive associations for nitrate intake and both papillary (RR = 2.10; 95% CI: 1.09–4.05; *p*-trend = 0.05) and follicular thyroid cancer (RR = 3.42; 95% CI: 1.03–11.4; *p*-trend = 0.01) among men.

Gatseva & Argirova (2005) studied the iodine status of school children between the ages of 11 and 14 years from 2 villages in Bulgaria living in areas with high nitrate levels in water. Their results showed statistically significant differences between the median urinary iodine levels of the total number of exposed (179.0 microg/l) and non-exposed (202.50 microg/l) children. The relative risk for the children exposed to high nitrate levels in drinking water, expressed as the odds ratio, was 8.145. They found a statistically significant difference for the prevalence of goiter among the exposed and non-exposed children. The results of the study confirmed the role of high nitrate levels in drinking water as a health risk factor for thyroid dysfunction.

Morris (2010) investigated the relationship between water chemistry and goiter development in two species of bamboo shark, *Chiloscyllium*spp. The results indicated that nitrate exposure did not affect growth rates (e.g., weight, length, and condition factor), nor did it decrease circulating plasma T4 concentrations during the 29 day experimental period. However, histological analysis of the thyroid glands in nitrate-exposed sharks did demonstrate the development of diffuse hyperplastic goiter. The results of his study support concerns that environmental nitrate exposure, in the absence of other factors, may be goitrogenic.

Eskiocak *et al.* (2005) investigated the chronic effects and the dose-response relationship of nitrate intake on thyroid functions in rats. According to their findings, histomorphological changes were observed in the 250 and 500 mg/l nitrate intake groups. These findings suggested that nitrate impairs thyroid function involving the hypothalamo-hypophysio-thyroidaxis.

Another study by Dhanwal (2011) reported a significant effect of increased phosphorous on thyroid hormones on tissue phosphate metabolism and renal phosphate handling. The effect led to increased filtered load of phosphorous causing hyperthyroidism. Generally, the positive relationship between inorganic ions in drinking water and goiter incidence is explained by Zoeller (2007). The researcher attributed it to competitive inhibition of iodide transport leading to decreased thyroid hormone secretion, followed by an increase in thyroid-stimulating hormone.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The research was conducted in Nandi Hills ward, Nandi County. The area is an urban settlement in Nandi County, Kenya. It is located in a highland area of lush green rolling hills at the edge of the Great Rift Valley in the southwestern part of Kenya, approximately 303 kilometres, by road, northwest of Nairobi. The coordinates of Nandi Hills, are: 0°06'01.0"N, 35°10'35.0"E (Latitude: 0.100278; Longitude: 35.176389). Nandi Hills lies at an elevation of approximately 2,047 metres (6,716 ft), above sea level (Globalfeed, 2015) and has a cool and wet climate with two rain seasons during the equinoxes. Temperatures vary between 18°C and 24°C which coupled with the rich volcanic soils make the area ideal for growing tea (KIG, 2014). Tea production in the Nandi Hills area uses high concentration of nitrogenous fertilizer. Nitrates can be discharged to water bodies through leaching and surface run-off and ends up in surface and ground water sources causing pollution (Maghanga *et al.*, 2012).

3.2: Population and Sample size

According to census 2009, the study area has a population size of 33, 545 people. The target population of the study comprised of only people who use borehole and stream water as sources of drinking water in Nandi Hills. From the target population, a total of 50 households were randomly sampled to obtain water, urine and table salts samples.

3.3 Sampling and Sample Collection

From the 50 households sampled in section 3.2, sampling was done by dividing the area into five points (four extreme ends and the centre). Fifty questionnaires were self-administered to the respondents. The urine and water samples were analyzed to determine the concentration of nitrates and phosphates while the table salt samples were analyzed to determine the content of iodine.

3.3.1 Collection of Water Samples

A total of 50 water samples were collected from both surface water (stream) and ground water (boreholes) sources using plastic bottles. Out of the 50 samples collected, 40 of them were from boreholes while 10 were from streams. The samples collected were kept in a cooler containing ice and transported to the laboratory within a period of 24 hours. This was done to inhibit metabolic processes of microbes and biodegradation reactions that could significantly change the levels of nitrates and phosphates.

3.3.2 Collection of Urine Samples

From the 50 households, 50 samples of first morning urine were obtained in labelled sample cups. This was achieved by first explaining to the respondents the purpose of the study. Also, the researcher was accompanied by public health officer who assisted in urine sample collection. The sample cups were kept in cooler containing ice at 4^0 C and transported to the laboratory within a period of 24 hours.

3.3.3 Collection of Table Salt Samples

From the 50 households identified, 50g of table salt were collected from each household alongside urine samples. Collection was biased to the salt stored in the household containers. The table salt samples were stored in transparent labeled zipped lock bags. The samples were transported to the laboratory within a period of 24 hours and analyzed.

3.4 Laboratory Analysis of the Samples

3.4.1 Analysis of Nitrate concentration in water and urine

Analysis of nitrates was done using cadmium reduction method according to Doane & Horwath (2003). In this case, the sample was filtered using a filter paper, and passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediaminedihydrochloride to form a highly colored azo dye which is measured colorimetrically using UV-Vis spectrometer. An absorbance measurement was made at 220 nm and corrected by subtracting a second measurement at 275 nm. This was done to compensate for the presence of organics.

3.4.2 Analysis of Phosphates Concentration in water and urine

Spectrophotometric method as described by Doane & Horwath (2003) was employed in the analysis. The method involves the formation of molybdophosphoric acid, which is reduced to the intensely colored complex, molybdenum blue. This analytical method is usually extremely sensitive and is reliable down to concentration of 0.1mg of phosphorus per liter of water (Aspila *et al.,* 1976).

Preparation of Standard Solutions: A 5point calibration curve was prepared with concentrations ranging from 0 – 5mg/L Phosphate from the stock standard solution. The standard solutions and the blank was treated according to the following “color development" procedure of Gale (2012). After measuring the absorbance of these solutions, a plot of absorbance versus concentration was generated.

Color Development in Sample: A 25 mL water sample to be analyzed was placed in an Erlenmeyer flask (measured with a volumetric pipet). Approximately 1.0 mL of ammonium molybdate solution was added and the mixture swirled for 10 minutes to mix properly. To the flask, two drops of stannous chloride solution was added and mixed by swirling. Development of a blue color within five minutes denoted the presence of phosphate ions. The measurements were recorded appropriately for each sample at 650 nm.

3.4.3 Analysis of Iodine Concentration

The concentration of Iodine in the table salt samples was determined using both titration and salt-pad methods.

3.4.3.1 Titration method

Ten grams of iodated salt was dissolved in 50 ml distilled water. Addition of sulfuric acid (1–2 ml) and potassium iodide (5 ml) was done, which in the presence of iodine a yellow color was obtained. The reaction mixture was then kept in a dark place for 10 minutes to reach the optimal reaction time, prior to titration with sodium thiosulfate using 2ml of starch as the indirect indicator. The concentration of iodine in salt was calculated based on the titrated volume (burette reading) of sodium thiosulfate according to the formula below as described by Jooste & Strydom (2010).

mg/kg (ppm) iodine = titration volume in ml x 21.15 x normality of sodium thiosulfate x 1000 / salt sample weight in g

3.4.3.2 Salt PAD Method

The salt PADs for analysis were obtained from the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, USA. The salt PAD is a paper card printed with hydrophobic wax barriers that define 12 reaction areas. All of the reagents needed to perform a single point of an iodometric titration are stored in the reaction area. A test solution was made by mixing the 3.25g salt sample with 15ml of distilled water. Shaking was done until the salt completely dissolved in the water. Three drops of the solution were applied to each of 12 reaction areas on the test card. A photograph of the test card was finally taken. An automated image analysis program was used to interpret the colour response by comparing the card to the test card visually stored images of test cards run with standard samples.

3.5 Ethical Considerations

Prior to urine sample collection, the participants were asked not to give their names thus maintaining their privacy and anonymity. The researcher also obtained all the required permits from Ministry of Education, National Commission for Science, Technology and Innovation (NACOSTI) and Ministry of Public Health and Sanitation before commencing the research. In the field, the researcher was accompanied by public health officers who explained the purpose of the research to the respondents before they gave out urine samples. No samples were collected from unwilling respondents.

3.6 Data Analysis

Data was analyzed descriptively using Statistical Package for Social Science (SPSS), version 20. The differences among mean concentrations of phosphates and nitrates in urine and water samples was determined using Analysis of variance (ANOVA). Difference between mean concentrations of iodine in table salt and WHO guidelines established using t-test analysis. In addition, Pearson correlation test was carried out to determine the association between the concentration of phosphate ions, nitrate ions, iodine ions and prevalence of goiter. Findings were presented using graphs, tables and charts.

CHAPTER FOUR

RESULTS

4.1 Concentrations of nitrate ions and phosphate ions in drinking water samples collected from streams and wells

4.4.1 Concentration of nitrate ions in streams water

Analysis of variance (ANOVA) results on differences in mean concentration of nitrate ions among streams and sampling points are summarized in Table 4.1 below. Mean concentration of nitrate ions among the three sampling points differed significantly ($p<0.05$). However, there was no significant difference ($p>0.05$) in nitrate ions concentration among the three streams. In addition, the interaction between stream and sampling point was insignificant ($p>0.05$).

Table 4.1: Effect of stream, sampling point and their interaction on Nitrate ions concentration

Source of variation	F-Value	P- Value
Stream (S)	14.671	0.542
Sampling point (SP)	8.966	0.031**
Stream× Sampling point (S×SP)	37.421.	0.067

****** Denotes significance at P<0.05*

From Table 4.2 below, the mean concentration for nitrate ions increased downstream in all the three streams. In the first stream, concentration of nitrate ions increased significantly (p<005) from 2.47 upstream to 3.35mg/l downstrem. Similarly, the concentration of nitrate ions increased significantly from 2. and 2.97 mg/l upstream to 3.17 and 3.46mg/l downstream in the second and third stream respectively.

Table 4. 2: Mean concentration of nitrate ions (mg/l) in Stream water

Stream	**Sampling point**	**Nitrate Mean± SE (mg/L)**
Stream 1	Upstream	2.47^{a}±0.044
	Midstream	2.53^{a}±0.031
	Downstream	3.35^{b}±0.012
Stream 2	Upstream	2.59^{a}± 0.041
	Mid-stream	2.64^{a}±0.076
	Downstream	3.17^{b}±0.092
Stream 3	Upstream	2.97^{a}±0.051
	Midstream	3.05^{a}±0.046
	Downstream	3.46^{b}±0.089

Mean values with different letters in a column are significantly different at p<0.05

4.1.2 Concentration of phosphate ions in stream water

Like nitrate ions concentration, the mean concentration of phosphate ions among the three sampling points differed significantly (p<0.05). However, there was no significant difference (p>0.05) in the concentration of phosphate ions among the three streams. Furthermore, there was insignificant (p>0.05) interaction between stream and sampling point as shown in Table 4.3 below.

Table 4.3: Effect of stream, sampling point and their interaction on the concentration of phosphate ions

Source of variation	**F-Value**	**P- Value**
Stream (S)	2.416	0.236
Sampling point (SP)	1.730	0.015**
Stream× Sampling point (S×SP)	14.120.	0.214

*** denotes significance at p<0.05*

Generally, results in Table 4.4 recorded an increase in the concentration of phosphate ions downstream among the three streams. Basically in stream 1, 2 and 3, phosphates ions ranged between 0.19-0.37, 0.2-0.39 and 0.27-0.40 mg/L respectively.

Table 4.4: Mean concentration (mg/l) of phosphate ions in the Stream water

Stream	Sampling point	Phosphate ions Mean± SE (mg/L)
Stream 1	Upstream	0.19a±0.01
	Midstream	0.31^{b}±0.08
	Downstream	0.37^{b}±0.12
Stream 2	Upstream	0.20^{a}± 0.01
	Mid-stream	0.34^{b}±0.06
	Downstream	0.39^{b}±0.09
Stream 3	Upstream	0.27^{a}±0.03
	Midstream	0.29^{a}±0.04
	Downstream	0.40^{b}±0.08

Mean values with different letters in a column are significantly different at p<0.05

4.1.3: Nitrate and phosphate concentrations in well water

The mean phosphate and nitrate concentrations in borehole water were 0.298 and 1.697 mg/L respectively (Table 4.5). Generally, nitrate levels were higher than phosphate levels in borehole water.

Table 4. 5: Means of concentrations of nitrate ions and phosphate ions in borehole water

Concentration of inorganic in water (mg/L)	
Phosphate ions	Nitrate ions
0.29±0.07	1.69±0.79

Figure 4.1 below clearly indicates that concentrations of phosphate ions in both borehole and stream water were within WHO acceptable limits (0.3 mg/L). Borehole water recorded phosphate ions that was slightly below the WHO acceptable limits

(0.29 mg/L). On the other hand, stream water recorded a mean value of 0.302 mg/l which was found to be just within the WHO guidelines.

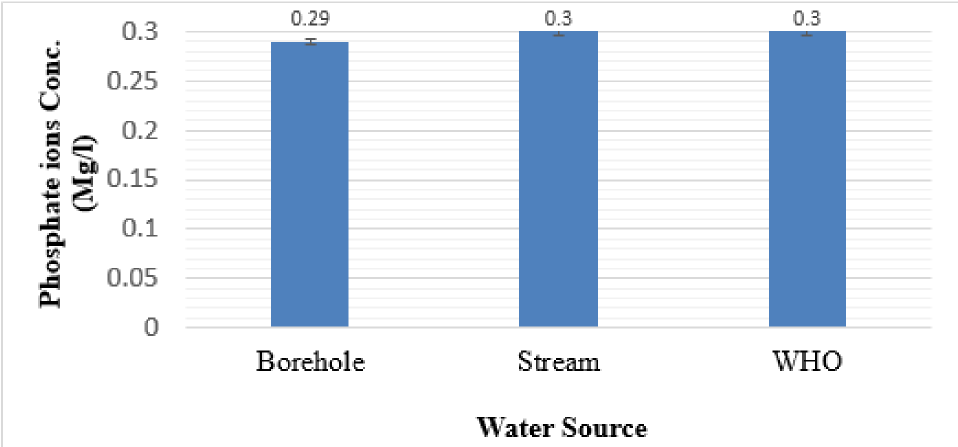

Figure 4. 1: Concentration of Phosphate ions in drinking and WHO acceptable limits

Figure 4.2 indicates that the concentrations of nitrate ions in both borehole and stream were within WHO acceptable limits (10 mg/L). About 1.69 and 2.91 mg/L of nitrate levels in borehole and stream water were recorded respectively.

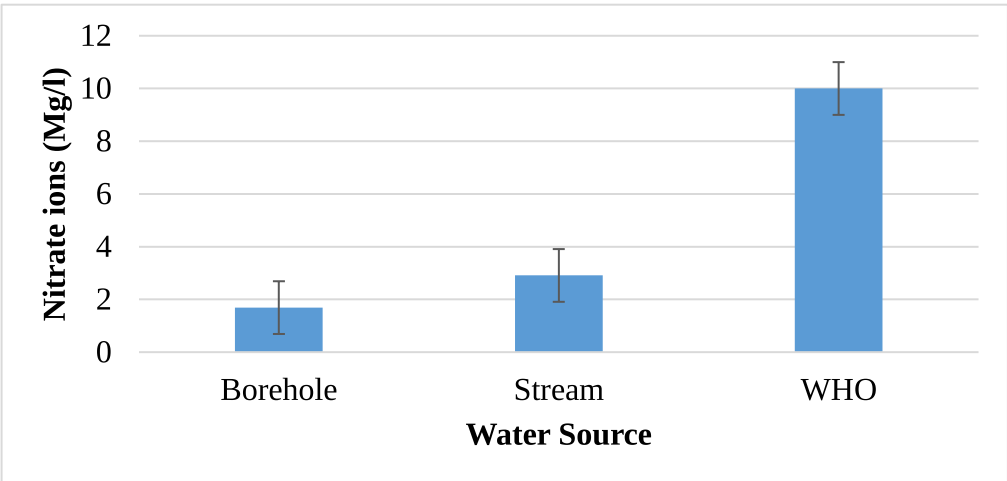

Figure 4. 2: Concentration of nitrate in drinking and WHO acceptable limits

4.2: Concentration of nitrates ions and phosphates ions in urine samples from selected inhabitants of the study area

For urine samples, mean concentrations for phosphates and nitrates are summarized in Table 4.6. Phosphate concentration was 0.539 mg/L while nitrate level was 709.59 mg/L.

Table 4.6: Means for nitrate and phosphate concentrations urine sample

Concentration of inorganic in urine (mg/L)	
Phosphate ions	Nitrates ions
0.539±0.30	709.59±5.57

4.3: Concentration of iodine ions in table salt from the inhabitants of the study area

From Table 4.7 below, titration method of iodine analysis recorded a lower mean concentration of 43.78 mg/L of iodine ions than salt PAD method which showed a mean concentration of 69.21 mg/L of iodine ions. Both levels of iodine ions were found to be WHO acceptable limit of 20-50mg/L.

Table 4.7: Mean concentration of Iodine

Iodine determination methods	**Mean±**
Titration	43.78±9.47a
Salt-pads	69.21±5.08b

Means with different letters are significantly different at p<0.05

Iodine determination with titration and salt PAD methods showed significant different results (P<0.05) as shown in Table 4.8.

Table 4.8: ANOVA on Effect of iodine determination methods

Source of Variation	Type III Sum of Squares	Df	Mean Square	F	Sig.
Iodine determination method	4654.518	2	623.259	41.648	0.03**

*** denotes significance at p<0.05*

4.4: Relationship between the concentration of nitrates-phosphates-iodine ions iodine in the samples and the prevalence of goiter in the study area

4.4.1: Demographic information

Gender and age formed the demographic variables in the present study. From 50 respondents in the study, majority of them were female (64%), while males were 36% as shown in Figure 4.3 below.

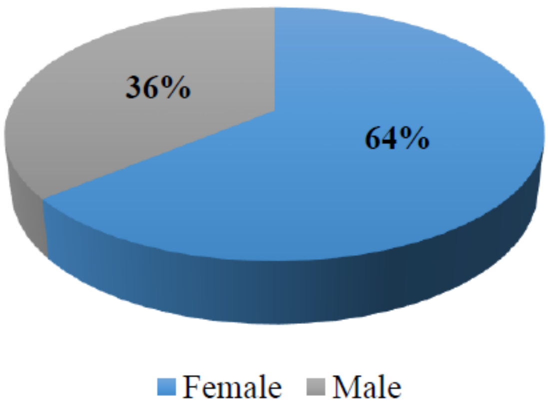

Figure 4. 3: Gender of the respondents

Results in figure 4.4 also revealed that majority of the respondents (38%) were between 31-40 years. Respondents between the age of 41-50, 18-30 and 51-60yrs were 34, 20 and 8% respectively.

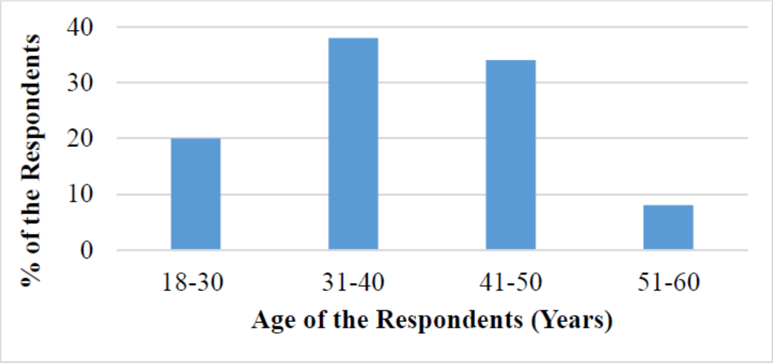

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Figure 4. 4: Age of the Respondents

4.4.2 Utilization of cooking salt by respondents

Results on how respondents in the study area used cooking salt are summarized in Table 4.9 below. Majority of the respondents (98%) agreed that they add salt into food while only 2% disagreed as shown in Table 4.9. Based on the type of salt used, most of the respondents (80%) used Kensalt, 16% used kaysalt while only 4% used others. Established on the stage at which salt was added, 40% of the respondents added at the final stage of cooking, 30% at the middle of cooking and 24% at early stages of cooking. Only 6% of the respondents added salt to food at the table. About 86% of the respondents kept salt in sealed containers while only 14% of them kept salt in open containers. Majority of the respondents (92%) had no substitution for table salt. However, 8% of them substituted the table salt with livestock salt.

Table 4.9: Table salt utilization among respondents

Addition of salt to food			
Yes	No		
49(98%)	1(2%)		
Type of salt used			
Kensalt	Kaysalt	Bahari	Others
40(80%)	8(16%)	0(0%)	2(4%)
Stage that salt was added			
Early	Mid	Final	On the table
12(24%)	15(30%)	20(40%)	3(6%)
If salt was kept in sealed containers			
Yes	No		
43(86%)	7(14%)		
Substitution of table salt			
Yes	No		
4(8%)	46(92%)		
Alternative sources of salt			
Livestock salt			
4(100%)			

From figure 4.5 below, 42%, 34%, 14% and 10% of the respondents used borehole, stream, rain and piped water for their general purposes respectively.

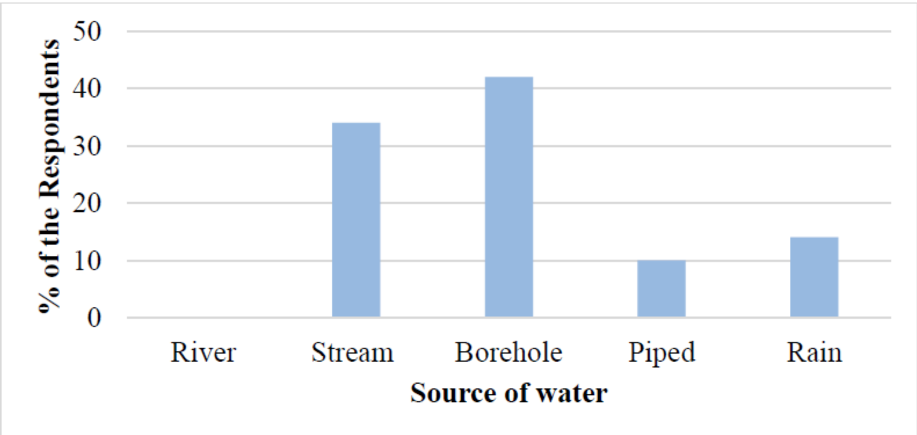


Figure 4. 5: Source of water for general purposes in the study area

Majority of the respondents (82%) used borehole water for drinking while only 18% of them used stream water as shown in Fig. 4.6.

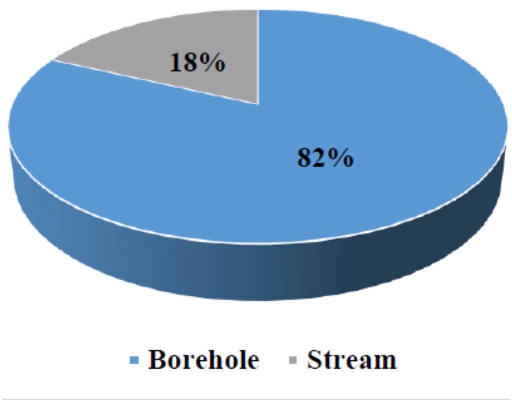


Figure 4. 6: Source of drinking water in the study area

As shown in Fig. 4.7 below, 54% of the respondents reported that at least one of their family members had suffered goiter while the remaining 46% had not suffered.

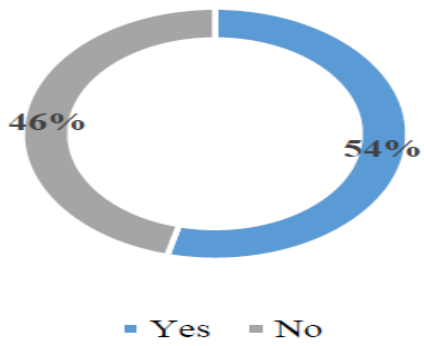


Figure 4. 7: Occurrence of goiter among respondents in the study area

From Table 4.10, the concentration of nitrate ions and phosphate ions in drinking water recorded an insignificant negative relationship ($p=0.936$; $r=-0.012$). On the other hand, the concentration of nitrate ions and phosphate ions in drinking water showed positive associations with prevalence of goiter. However, the associations were found to be insignificant ($p>0.05$).

Table 4.10:Relationship between nitrate- phosphate ions and prevalence of goiter in drinking water

		NO_3^-	PO_4^{3-}	Prevalence of Goiter
NO_3^-	Pearson Corr (r)	1	-.012	0.179
	Sig. (2-tailed) (P)		.936	0.218
PO_4^{3-}	Pearson Corr. (r)	-.012	1	0.201
	Sig. (2-tailed) (P)	.936		0.166

Correlation results in Table 4.11 showed that nitrate concentration had a significant positive association with prevalence of goiter ($r=0.734$; $P<0.05$). However, the concentration of phosphate ions in urine recorded an insignificant positive relationship with occurrence of goiter ($r=0.227$; $P>0.05$).

Table 4.11:Relationship between nitrate- phosphate ions and prevalence of goiter in urine samples

Variables		PO_4^{3-}	NO_3^-	Prevalence of goiter
PO_4^{3-}	Pearson Corr. (r)	1	0.240	0.227
	Sig. (2-tailed) (P)		0.136	0.159
NO_3^-	Pearson Corr.(r)	0.240	1	0.734
	Sig. (2-tailed) (P)	0.136		.000**

*** denotes significance at P<0.05*

Correlation results in Table 4.12 clearly indicated that iodine concentration in table salt used in households within the study area recorded a positive relationship ($r=0.275$) with goiter occurrence. However, the relationship was shown to be insignificant ($p>0.05$).

4.12: Relationship between iodine ions and prevalence of goiter in table salt

		Iodine ions	Prevalence of goiter
Iodine ions	Pearson Correlation (r)	1	0.275
	Sig. (2-tailed) (P)		0.071
Prevalence of goiter	Pearson Correlation (r)	0.275	
	Sig. (2-tailed) (P)	0.071	1

CHAPTER FIVE

DISCUSSION

5.1 Concentrations of nitrate ions and phosphate ions in stream and borehole water

5.1.1 Nitrate ions concentrations in borehole water

Water collected from boreholes in the study area recorded traces of nitrate ions that were found to be below the WHO acceptable limits. The traces of nitrate ions are borehole water is attributed to the fact that soil contains nitrate-rich rock minerals, which can dissolve gradually (Conway& Pretty, 2013). Another possible source of nitrate ions in borehole water in the region could be due to excessive use of chemical fertilizers by farmers given that tea farming is one of the main economic activities in the area. Furthermore, the depth of the boreholes in the study area could have contributed to the presence of nitrate ions in the water. According to Pulido-Bosch *et al.* (2000), shallow boreholes which draw water from intensively cultivated superficial formations, yield waters with high nitrate ions. The study further showed that when the boreholes are deeper and penetrate low-permeability cations in the superficial layers, the waters contain little nitrate ions. Similar results were also found in a study of groundwater quality by Munoz-Carpena *et al.* (2005) in South Florida agricultural area U.S.A. who showed that some nitrate concentrations were below the WHO acceptable limits of 10mg/L while others were above the limit. A study by Tay (2004) recorded NO^{3-}concentration ranging from 0.01 to 0.324 mg/L in ground water studied in the Ketu District. A study by Adekunle *et al.,* (2007) of groundwater quality in a typical rural settlement in Southwest Nigeria showed that Nitrate levels in some borehole water were within the WHO acceptable limits. Karikari & Ansa-Asare (2006) also recorded concentration ranging from <0.001 to 0.921mg/L in surface water in South Western Ghana.

5.1.2 Nitrate ions concentrations in stream water

The mean Nitrate values at all the sampling sites ranged between 0.19 mg/L and 0.40 mg/L. These levels were below the drinking water Nitrate guideline value set by WHO (2003), hence the streams in the study area were not nitrate polluted. However, the levels of nitrate recorded at the study area could be as a result of certain natural processes like decomposition of vegetation and activities of nitrogen fixing bacteria and precipitation (Conway& Pretty, 2013). Most importantly, the major causes of high nitrate concentration could be due to the farming activities that go on around the catchment of the study area. This is in line with the findings of Tomer *et al.* (2003), that elevated levels of nitrate is often noted in streams draining watersheds with high levels of corn production, nitrogen fertilizer application as well as runoffs from uncontained livestock operations. Furthermore, Bittner (2000) reported that after fertilizers are applied to fields, nitrogen not absorbed by crops nitrifies flows over the ground surface into the nearest stream. A study carried out by Biggs *et al.* (2004) reported levels of nitrate ions that were within WHO acceptable standards in the Piracicaba stream in Brazil.

The findings from the study also reported an increase in concentration of nitrate ions downstream among the three streams used in the study. This is attributed to increase in human pollution downstream. These results are in harmony with others studies. Takem *et al.* (2010) reported that the levels of nitrate ions in the downstream and upstream ranged between 24 and 21 mg/L respectively. Furthermore, Mladenov *et al.* (2005) reported average NO^{3-} being more than two times higher in the downstream than upstream of Notwane stream in south- eastern Botswana. Earlier on, Olajire and Imeokparia (2001) reported NO_3 levels in the Osun stream in Nigeria to range between 20 and 14 mg/L downstream and upstream respectively.

5.1.3 Concentration of Phosphate ions in Borehole water

Phosphate concentrations in drinking water from borehole recorded a mean of 0.298 mg/L. The low levels of phosphates ions in borehole water could be due to the fact that phosphate tends to sorb to soil and aquifer sediments and is not readily transported in groundwater (Holman *et al.,* 2008). However, the presences of phosphate ion traces in borehole water within the study area could be attributed to what Salvato *et al.* (2003) explained as excessive use of chemical fertilizers in agriculture. In addition, Tjandraatmadja *et al.* (2010) proposed; cleaning products, cosmetics, medicated shampoos, food products, faeces and urine as sources of phosphate in groundwater. The principal source of phosphate ions in borehole water is established during natural process of weathering, rocks gradually release phosphorus as phosphate ions which are soluble in water and gradually mineralize phosphate compounds breakdown. Findings from the study are in agreement with those recorded by Tay (2004) where the concentrations in ground water ranged from 0.01 to 0.3mg/l with a mean value of 0.11mg/L. Another study by Dubrovsky *et al.* (2010) reported that the estimated background concentration of orthophosphate for more than 400 shallow wells was 0.03 mg/L.

5.1.4 Concentration of phosphate ions in stream water in the study area

Concentration of phosphate ions in stream water was 0.302 mg/L. These traces of phosphates ions could have been brought about by excessive use of readily available conventional chemical fertilizers on tea farms in the study area. Levallois *et al.* (1998) recorded that use of chemical fertilizers is the major source of surface waters contamination. Apart from the use of chemical fertilizers, the use of detergents in car or cloth washing at the banks could be the possible source of high phosphate concentration in the stream. Furthermore, Brain (2011) established that during natural process of weathering, rocks gradually release phosphorus as phosphate ions which are soluble in water and gradually mineralize phosphate compounds breakdown.

Similar findings were reported by Ansa-Asare *et al.* (2006) where phosphate concentration ranged from <0.001 to 0.321mg/L in surface water in South Western Ghana.

5.2 Concentration of nitrate ions and phosphate ions on urine samples

Findings from the present study showed high concentrations of phosphate and nitrate ions urine samples (0.53 and 709.5 mg/L) respectively even after their levels in water were reported to be within acceptable limits. This suggested that either there could be other sources of these ions in the study area other than drinking water or the absorption of these ions is inhibited. WHO (2011) reported that the contribution of drinking-water to nitrate intake in human beings is usually less than 14%. Therefore, the levels could be attributed to nutritional sources of nitrate and phosphate ions. A study by Ohshima *et al.* (1994) **v**egetables and cured meat are in general the main sources of nitrate in the diet, but small amounts may be present in fish and dairy products. Another study further explained that several vegetables and fruits contain

200–2500 mg of nitrate per kilogram (van Duijvenboden & Matthijsen, 1989). The nitrate content of vegetables can be affected by processing of the food, the use of fertilizers and growing conditions, especially the soil temperature and (day) light intensity (WHO, 1995). Vegetables such as beetroot, lettuce, radish and spinach often contain nitrate concentrations above 2500 mg/kg, especially when they are cultivated in greenhouses (WHO, 2011).

The excess nitrate excretion (709.59mg/l) that was observed could have originated from endogenous synthesis which amounts in normal healthy humans to 1 mmol/day on average, corresponding to 62 mg of nitrate per day (WHO, 2011). Studies have further revealed that increased endogenous synthesis of nitrate in humans with induced infections and inflammatory reactions (Ohshima *et al.*, 1994). A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline, followed by oxidation of the nitric oxide to nitrous anhydride and then reaction of nitrous anhydride with water to yield nitrite. Nitrite is rapidly oxidized to nitrate through reaction with Hb (WHO, 2011).

5.3 Iodine concentration in table salt

Findings from the study recorded mean concentrations of 43.78-69.21mg/L when titration and salt PADs methods were used respectively. The difference in iodine concentration between the two methods could be to the inaccuracy of titration method. Jooste & Strydom (2010) reported th-at titration method is not efficient when a salt sample is fortified with potassium iodide, since the method will not detect iodine contents. Jooste & Strydom (2010) further recorded that although titration is the gold standard analytical method recommended for factory quality assurance and research studies, it is not practical for many small fortification facilities nor for field use in salt surveys. On the other hand, saltPAD method has been reported to be easy to use and accurate (Myers *et al.,* 2016).

The high concentration of iodine in table salt in the study area could be attributed salt handling practices in the area. For example, 66% of the respondents store salt in sealed containers. Zimmermann *et al*. (2008) reported that the factors which influence the stability of iodine in the household salts include the duration of the storage, the size of the crystals, impurities and moisture, the ambient temperature of the storage and the humidity and the sunlight exposure. Similar findings were reported by Ranganathan & Karmarkar (2006) where iodine levels of 30-50 ppm were found in 649 (36.0%) samples and 228 (12.6%) salt samples had iodine levels which were greater than 50ppm.

5.4 Relation between Nitrate ions, Phosphate ions, Iodine ions and Goiter occurrence in the study area

5.4.1 Relation between Nitrate-Phosphate ions in drinking water and Goiter occurrence in the study area

Findings in Table 4.10 recorded an insignificant positive relationship between nitrate-phosphate- iodine ions and goiter occurrence. Several studies on the association between inorganic components in drinking and goiter occurrence have focused on nitrate, fluoride, iodine and manganese ions (Fernando *et al.,* 2009). However, less has been done on phosphate ions. The results obtained in the present study are in contrast with several studies that have been carried out. Ward *et al.* (2010) recorded a significant association between nitrate intake from public drinking water supplies and incidence of thyroid cancer. Furthermore, studies by Van Maanen *et al.* (1994) ;Tajtakova *et al.* (2006) reported that populations with sufficient iodine intake provided some evidence that nitrate ingestion via drinking water was associated with subclinical hypothyroidism and hypertrophy of the thyroid. Earlier on, van Maanen *et al* (1994) described an association between high nitrate concentrations in drinking-water and goitre incidence. A dose–response relationship between inorganic ions in drinking water and goiter occurrence was demonstrated by Höring *et al.* (1991). Morris (2010) investigating on the relationship between water chemistry and goiter development in two species of bamboo shark, *Chiloscyllium*spp, reported a positive associated between the two variables.

A number of subsequent studies in Slovakia, Bulgaria, Germany and the USA have reported a correlation between various measures of nitrate intake and effects on thyroid function, but all suffer from methodological and data problems that preclude definitive conclusions (Tajtakova *et al.,* 2006; Radikova *et al.,* 2008; Ward *et al.,* 2010). Dhanwal (2011) reported a significant effect of increased phosphorous on thyroid hormones on tissue phosphate metabolism and renal phosphate handling. The effect led to increased filtered load of phosphorous causing hyperthyroidism. The positive relationship between inorganic ions in drinking water and goiter incidence is explained by Zoeller (2007). The researcher attributed it to competitive inhibition of iodide transport leading to decreased thyroid hormone secretion, followed by an increase in thyroid-stimulating hormone.

On the other hand, insignificant relationship between inorganic ions in water and goiter occurrence in the present study could be attributed to some reasons. Firstly, the concentrations of both phosphate and nitrate in borehole and stream water were within the recommended limits by WHO. Secondly, it could be due to the season in which the water samples were collected. Finally, most of the studies that have reported positive association were done on laboratory animals while the present study used humans, hence the difference. Even though, results of the present study are in agreement with other studies. A clinical study in the Netherlands, did not find any relationship between nitrate intake and thyroid structure or function (Blount *et al.,* 2009). Furthermore, Fernando *et al.* (2009) reported an insignificant positive relationship between inorganic ions in drinking water and goiter occurrence in Sri Lanka. In addition, other studies (Crow *et al.,* 1998; Zaki *et al.,* 2004; Eskiocak *et al.,* 2005) have demonstrated insignificant relationship between inorganic components of water and goiter occurrence. However, these studies reported that chronic exposure (≥ 5 years) to nitrate ions and phosphate ions could result in the development of diffuse colloid goiter

5.4. Relation between Iodine in table salt and Goiter occurrence in the study area

Findings from the study recorded an insignificant positive relationship between iodine concentration in table salt and goiter occurrence in the study area. This could be attributed to the fact that the concentration of iodine in salt was found to be within the acceptable range of 20-50 mg/L. In populations with sufficient iodine intake, the prevalence of goiter in is usually less than 5% (WHO, 2011). However, the occurrence of goiter in the area could be attributed to post production factors like handling of salt during cooking and storage that could alter iodine level in the long run. For example, 14% of the respondents do not store salt in sealed containers. This could have led to iodine loss due to alteration of the moisture content, acidity/alkalinity of salts. Also the stage in which salt was added into the food could have contributed to iodine loss. In the present study 24% of the respondents added salt at the early stage of cooking. This could lead to overcooking of iodine hence loss of it. These findings are in agreement with those for Shawel *et al.* (2010). The researchers reported that iodine content was significantly lost through post production factors. Among the factors, handling of salt during and after cooking was reported to be a significant factor.

Furthermore, a study conducted by Kapil (1998) in Delhi documented that there was about 31% iodine loss from iodized salt when exposed to heat for long. A study done in Ethiopia also indicated that exposure to sunlight was associated with loss of iodine level in salt (Gebremariam *et al.*, 2013). This might be due to the effect of heat on the iodine content since the halogen iodide over time and exposure to excess oxygen and carbon dioxide slowly oxidizes to metal carbonate and elemental iodine which then evaporates (Waszkowiak & Szymandera, 2008).

Salt storage at home was significantly associated with availability of adequately iodized salt. A study conducted in London showed that duration and mode of salt storage had significant impact on the level of iodine (Oshinowo *et al.*, 2004; Waszkowiak & Szymandera, 2008). This might be due to the effect of physical or environmental factors like moisture content of the salt, humidity of the atmosphere, light and weather condition.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Both the concentration of nitrate ions and phosphate ions in borehole and stream water were within WHO acceptable limits, suggesting that the water sources in the study area are less polluted. There was significant difference in concentration of nitrate ions and phosphate ions among different sampling points in the three streams used in the study, suggesting that pollution of stream water in the study area increases downwards.

The levels of nitrate ions in urine samples were higher than the acceptable limits (100-200 mg/L). This suggests that either there could be other sources of nitrate ions or the absorption of the same is inhibited. However, the concentration of phosphate ions was below the acceptable limits (25-45 mg/L).

The level of iodine in table salt was found to be within WHO acceptable limit of 20-50 mg/L. Therefore, the study concludes that table salt used in the study area is iodized and the post production loss of iodine is minimal.

The relationship between nitrate, phosphate and iodine ions and goiter occurrence in water was insignificant, concluding that the occurrence of goiter in the study area is not dependent on the ions tested in the study. Hence, there could be other factors that contribute to the prevalence of goiter in the study.

6.2 Recommendations

Based on the result obtained from the present study, the following recommendations are made:

Use of both borehole and stream water for drinking in the study area should continue. Also for stream water, upstream water should be used for drinking since the level of inorganic ions is low.

The present study was limited to the association between nitrate-phosphate-iodine ions and goiter. Therefore, it is recommended that further studies to be done so as to relate other inorganic ions like fluoride ions, magnesium ions or calcium ions in drinking water with goiter occurrence in the study area.

Since the present study did not examine the influence of seasonal variation on nitrate-phosphate levels, the results obtained may not reflect full information on the extent of nitrate and phosphate ions in boreholes and streams water in the study area. Therefore, there is need for detailed studies based on seasonal variation and complete analysis.

REFERENCES

Adekunle, I. M., Adetunji, M. T., Gbadebo, A. M., & Banjoko, O. B. (2007). Assessment of groundwater quality in a typical rural settlement in Southwest Nigeria. *International journal of environmental research and public health*, *4*(4), 307-318.

Al-Masri, M.S., Amin, Y., Ibrahim, S., Al-Bich, F. 2004. *Distribution of some trace metals in Syrian phosphogypsum. Applied Geochemistry*,19: 747-753

Andersson, M., de Benoist, B., & Rogers, L. (2010). Epidemiology of iodine deficiency: salt iodisation and iodine status. *Best Practice & Research Clinical Endocrinology & Metabolism*, *24*(1), 1-11.

Andersson, M., Karumbunathan, V., & Zimmermann, M. B. (2012).Global iodine status in 2011 and trends over the past decade.*The Journal of nutrition*, *142*(4), 744-750.

Armarego, W. L., & Chai, C. L. L. (2013).*Purification of laboratory chemicals*.Butterworth-Heinemann.

Aschebrook-Kilfoy, B., Shu, X. O., Gao, Y. T., Ji, B. T., Yang, G., Li, H. L., ...& Ward, M. H. (2013). *Thyroid cancer risk and dietary nitrate and nitrite intake in the Shanghai women's health study*. *International Journal of Cancer*, *132*(4), 897-904.

Ashraf, A., Amini, M., &Hovsepian, S. (2010).*Prevalence of goiter in Isfahan, Iran, fifteen years after initiation of universal salt* iodization.*Journal of health, population, and nutrition*, *28*(4), 351.

Ashurst, J., & Wasson, M. (2011).Methemoglobinemia: *a systematic review of the pathophysiology, detection, and treatment*. *Delaware medical journal*, *83*(7), 203-208.

Aspila, K. I., Agemian, H., & Chau, A. S. Y. (1976*). A semi-automated method for the determination of inorganic, organic and total phosphate in sediments*. *Analyst*, *101*(1200), 187-197.

Bahn, R. S., & Castro, M. R. (2011).*Approach to the patient with nontoxic multinodulargoiter.The Journal of Clinical Endocrinology & Metabolism*, *96*(5), 1202-1212.

Bajpai D, Tyagi VK (2007). *Laundry detergents: an overview*. 56: 327-340.

Barrett, K. E., Barman, S. M., &Boitano, S. (2010). *Ganong's review of medical physiology*. New Delhi: McGraw Hill, 2010.

Beckey, H. D. (2013). *Principles of Field Ionization and Field Desorption Mass Spectrometry: International Series in Analytical Chemistry*. Elsevier.

Berner, E. K., &Berner, R. A. (2012).*Global environment: water, air, and geochemical cycles*. Princeton University Press.

Bhatnagar, A., &Sillanpää, M. (2011).*A review of emerging adsorbents for nitrate removal from water.Chemical Engineering Journal*, *168*(2), 493-504.

Biggs, T. W., Dunne, T., & Martinelli, L. A. (2004). *Natural controls and human impacts on stream nutrient concentrations in a deforested region of the Brazilian Amazon basin. Biogeochemistry,* 68(2), 227-257.

Bittner, A. (2000). *Nepal drinking water quality assessment: Nitrates and ammonia* (Doctoral dissertation, Massachusetts Institute of Technology).

Blount, B. C., Rich, D. Q., Valentin-Blasini, L., Lashley, S., Ananth, C. V., Murphy, E., ... & Hore, P. (2009). Perinatal exposure to perchlorate, thiocyanate, and nitrate in New Jersey mothers and newborns. *Environmental science & technology*, *43*(19), 7543-7549

Boelaert, K., Newby, P. R., Simmonds, M. J., Holder, R. L., Carr-Smith, J. D., Heward, J. M., ... & Franklyn, J. A. (2010). Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease.*The American journal of medicine*, *123*(2), 183-e1.

Bryan, N. S., Alexander, D. D., Coughlin, J. R., Milkowski, A. L., &Boffetta, P. (2012). Ingested nitrate and nitrite and stomach cancer risk: an updated review. *Food and Chemical Toxicology*, *50*(10), 3646-3665.

Burow, K. R., Nolan, B. T., Rupert, M. G., &Dubrovsky, N. M. (2010). Nitrate in groundwater of the United States, 1991− 2003. *Environmental science & technology*, *44*(13), 4988-4997.

Chand, S., Ashif, M., Zargar, M. Y., & Ayub, B. M. (2011). *Nitrate pollution: a menace to human, soil, water and plant. Universal Journal of Environmental Research and Technology*, *1*, 22-32.

Chapra, S.C., Pelletier, G.J., Tao, H., 2008. QUAL2K: *a Modeling Framework for Simulating River and Stream Water Quality,* Version 2.11:

Conway, G. R., & Pretty, J. N. (2013). *Unwelcome harvest: agriculture and pollution*. Routledge.

Conway, Gordon R., and Jules N. Pretty. *Unwelcome harvest: agriculture and pollution*. Routledge, 2013.

Corbridge, D. E. (2013).*Phosphorus: chemistry, biochemistry and technology*. CRC Press.

Crow, G. L., Atkinson, M. J., Ron, B., Atkinson, S., Skillman, A. D., & Wong, G. T. (1998). *Relationship of Water Chemistry to Serum ThyroidHormones in Captive Sharks with Goitres. Aquatic Geochemistry*, *4*(3-4), 469-480.

Dhanwal, D. K. (2011). *Thyroid disorders and bone mineral metabolism. Indian journal of endocrinology and metabolism*, *15*(6), 107.

Doane, T. A., & Horwath, W. R. (2003). *Spectrophotometric determination of nitrate with a single reagent*. *Analytical Letters*, *36*(12), 2713-2722.

Dubrovsky, N. M., Burow, K. R., Clark, G. M., Gronberg, J. M., Hamilton, P. A., Hitt, K. J., ... & Rupert, M. G. (2010). *The quality of our Nation's waters-Nutrients in the Nation's streams and groundwater, 1992-2004* (No. 1350). US Geological Survey.

Eskiocak, S., Dundar, C., Basoglu, T., & Altaner, S. (2005). *The effects of taking chronic nitrate by drinking water on thyroid functions and morphology. Clinical and experimental medicine*, *5*(2), 66-71.

Eskiocak, S., Dundar, C., Basoglu, T., &Altaner, S. (2005).*The effects of taking chronic nitrate by drinking water on thyroid functions and morphology*.*Clinical and experimental medicine*, *5*(2), 66-71.

Fagman, H., & Nilsson, M. (2010).*Morphogenesis of the thyroid gland.Molecular and cellular endocrinology*, *323*(1), 35-54.

Fernando, R., Mettananda, D. S. G., & Kariyakarawana, L. (2009). I*ncidental occult carcinomas in total thyroidectomy for benign diseases of the thyroid. Ceylon Medical Journal*, *54*(1).

Fewtrell L. 2004. *Drinking Water Nitrate, Methemoglobinemia, and Global Burden of Disease: A Discussion.Environ Health Perspect.* 2004 Oct; 112(14): 1371–1374.

Förstner, U., &Wittmann, G. T. (2012).*Metal pollution in the aquatic environment*.Springer Science & Business Media.

Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., ...& Voss, M. (2013). *The global nitrogen cycle in the twenty-first century*.*Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *368*(1621), 20130164.

Gale, R. J. (Ed.). (2012). *Spectroelectrochemistry: theory and practice*. Springer Science & Business Media.

Gatseva, P. D., &Argirova, M. D. (2005). *Iodine status of children living in areas with high nitrate levels in water. Archives of environmental & occupational health*, *60*(6), 317-319.

Gebremariam, H. G., Yesuf, M. E., & Koye, D. N. (2013). *Availability of adequately iodized salt at household level and associated factors in Gondar town, Northwest Ethiopia. ISRN Public Health*, *2013*.

Georgantas DA, Grigoropoulou HP (2007)."*Phosphorus Removal from Synthetic Wastewater Using Spent Alum Sludge",8th International Conference on Environmental Science and Technology, Lemnos island, Greece*, 8 – 10 September.

Globalfeed (2015). *Road Distance Between Nairobi And Nandi Hills With Interactive* Map. Retrieved from http://distancecalculator.globefeed.com/ on 3rd August 2015

Goel P.K., 2006. *Water Pollution-Causes, Effects and Control, New Delhi: New Age International*. P.179-80.

Golterman, H. L. (2007). *The chemistry of phosphate and nitrogen compounds in sediments. Springer Science & Business Media.*

Greer, F. R., M. Shannon, C. o. Nutrition, and C.o. E. Health. 2005. *Infant Methemoglobinemia: The Role of Dietary Nitrate in Food and Water. Pediatrics* 116:784-786.

Haymart, M. R., Banerjee, M., Stewart, A. K., Koenig, R. J., Birkmeyer, J. D., & Griggs, J. J. (2011*). Use of radioactive iodine for thyroid cancer.Jama*, *306*(7), 721-728.

Hemminki, K., Li, X., Sundquist, J., &Sundquist, K. (2010). *The epidemiology of Graves' disease: evidence of a genetic and an environmental contribution*. *Journal of autoimmunity, 34*(3), J307-J313.

Höring, H., Nagel, M., & Haerting, J. (1991). *Das nitratbedingte Strumarisiko in einem Endemiegebiet. In Quantitative Methoden in der Epidemiologie* (pp. 147-153). Springer Berlin Heidelberg.

Hughes, K., & Eastman, C. (2012).Goiter: *Causes, investigation and management*. *Australian family physician*, *41*(8), 572.

Hungate, R. E., R. W. Dougherty, et al. (2013). *Microbial and physiological changes associated with acute indigestion in sheep.* Cornell Veterinarian 2: 423-449.

Hwang, Y. H., Kim, D. G., & Shin, H. S. (2011). *Mechanism study of nitrate reduction by nano zero valent iron. Journal of Hazardous Materials*, *185*(2), 1513-1521.

Jadhao, R. B. (2013*). Contamination Of Water By Nitrate: A General Discussion Problems Associated, Causes, Prevention And Effects. International Journal of Innovative Research and Development*, *2*(7).

Jankovic, B., Le, K. T., &Hershman, J. M. (2013). *Hashimoto's thyroiditis and papillary thyroid carcinoma: is there a correlation?.The Journal of Clinical Endocrinology & Metabolism*, *98*(2), 474-482.

Jenkyns, H. C. (2010). *Geochemistry of oceanic anoxic events.Geochemistry, Geophysics, Geosystems*, *11*(3).

Jooste, P. L., & Strydom, E. (2010). *Methods for determination of iodine in urine and salt. Best Practice & Research Clinical Endocrinology & Metabolism*, *24*(1), 77-88.

Kapil, U. (1998). *Current status of iodine deficiency disorders control program*. *Indian pediatrics*, *35*, 831-840.

Karikari, A. Y., & Ansa-Asare, O. D. (2006). *Physico-chemical and microbial water quality assessment of Densu River of Ghana. West African Journal of Applied Ecology*, *10*(1).

Karikari, A. Y., & Ansa-Asare, O. D. (2006*). Physico-chemical and microbial water quality assessment of Densu River of Ghana. West African Journal of Applied Ecology*, *10*(1).

KIG.(2014). "*Overview of Nandi Count*y".© 2014 KenyaInformationGuide.com (KIG). Retrieved August 3, 2015

Kilfoy, B. A., Zhang, Y., Park, Y., Holford, T. R., Schatzkin, A., Hollenbeck, A., & Ward, M. H. (2011).*Dietary nitrate and nitrite and the risk of thyroid cancer in the NIH‐AARP Diet and Health Study*.*International Journal of Cancer*, *129*(1), 160-172.

Kuzelka, R. D., &Ennenga, W. (Eds.). (2013). *Nitrate contamination: exposure, consequence, and control* (Vol. 30). Springer Science & Business Media.

Levallois, P., Theriault, M., Rouffignat, J., Tessier, S., Landry, R., Ayotte, P., ... & Chiasson, C. (1998). *Groundwater contamination by nitrates associated with intensive potato culture in Quebec. Science of the Total Environmen*t, 217(1), 91-101.

Li, M., & Eastman, C. J. (2012).*The changing epidemiology of iodine* deficiency.*Nature Reviews Endocrinology*, *8*(7), 434-440.

Maghanga, J. K., Kituyi, J. L., Kisinyo, P. O., &Ng'etich, W. K. (2012*).Impact of nitrogen fertilizer applications on surface water nitrate levels within a Kenyan* tea plantation.*Journal of Chemistry, 2013*.

Majumdar, D. and N. Gupta, 2000. *Nitrate pollution of groundwater and associated human health disorders. Indian J. Environ. Health*, 42: 28-39.

Martin, K. J., & González, E. A. (2011). *Prevention and control of phosphate retention/hyperphosphatemia in CKD-MBD: what is normal, when to start, and how to treat*?.*Clinical Journal of the American Society of Nephrology*, *6*(2), 440-446.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). *Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agricultur*e. *Annals of Botany*, mcq028.

Memon, W., Khanzada, T. W., Samad, A., & Kumar, B. (2010).*Incidence of thyroid carcinoma in multinodulargoiters.Rawal Medical Journal*, *35*(1), 65-67.

Menconi, F., Marcocci, C., &Marinò, M. (2014).*Diagnosis and classification of Graves' disease*.*Autoimmunity reviews*, *13*(4), 398-402.

Mladenov, N., McKnight, D. M., Wolski, P., & Ramberg, L. (2005*). Effects of annual flooding on dissolved organic carbon dynamics within a pristine wetland, the Okavango Delta, Botswana. Wetlands*, *25*(3), 622-638.

Morris, A. L. (2010). *The Relationship Between Water Chemistry And Goiter Development In Two Species Of Bamboo Shark, Chiloscylliumspp* (Doctoral dissertation, University of Florida).

Muñoz-Carpena, R., Ritter, A., & Li, Y. C. (2005). *Dynamic factor analysis of groundwater quality trends in an agricultural area adjacent to Everglades National Park*. *Journal of Contaminant Hydrology*, *80*(1), 49-70.

Myers, N. M., Strydom, E. E., Sweet, J., Sweet, C., Spohrer, R., Dhansay, M. A., & Lieberman, M. (2016). saltPAD: *A New Analytical Tool for Monitoring Salt Iodization in Low Resource Settings. Nanobiomedicine*, *3*, 5.

Ogbera, A. O., & Kuku, S. F. (2011).Epidemiology of thyroid diseases in Africa.*Indian journal of endocrinology and metabolism*, *15*(Suppl2), S82.

Ohshima, H., Bandaletova, T. Y., Brouet, I., Bartsch, H., Kirby, G., Ogunbiyi, F., ... & Pipitgool, V. (1994). *Increased nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase induced in hamsters infected with liver fluke* (Opisthorchis viverrini). Carcinogenesis, 15(2), 271-275.

Olajire, A. A., & Imeokparia, F. E. (2001). *Water quality assessment of Osun River: Studies on inorganic nutrients*. *Environmental Monitoring and Assessment*, *69*(1), 17-28.

Oshinowo, T., Diosady, L., Yusufali, R., & Laleye, L. (2004*). Stability of salt double-fortified with ferrous fumarate and potassium iodate or iodide under storage and distribution conditions in Kenya. Food and nutrition bulletin*, *25*(3), 264-270.

P.Rajesh, Kesavan Muthu S. Seshadri A.Manikandan T.Gnanasambandan And S. Gunasekaran 2015. *Physico-Chemical Characteristics of Ground Water at Pappankuppam (Tamil nadu), India.* International journal of current research and reviews.

Paschke, R., Hegedüs, L., Alexander, E., Valcavi, R., Papini, E., &Gharib, H. (2011). *Thyroid nodule guidelines: agreement, disagreement and need for future research. Nature Reviews Endocrinology*, *7(6), 354-361.*

Pazaitou-Panayiotou, K., Michalakis, K., &Paschke, R. (2012).Thyroid cancer in patients with hyperthyroidism.*Hormone and metabolic research Hormon-und Stoffwechselforschung Hormones etmetabolisme*, *44*(4), 255-262.

Pearce, E. N., &Braverman, L. E. (2009*).Environmental pollutants and the thyroid.Best Practice & Research Clinical Endocrinology & Metabolism*, *23*(6), 801-813.

Pulido-Bosch, A., Pulido-Leboeuf, P., Molina-Sánchez, L., Vallejos, A., & Martin-Rosales, W. (2000). *Intensive agriculture, wetlands, quarries and water management. A case study (Campo de Dalias, SE Spain). Environmental Geology*, *40*(1), 163-168.

Pulido-Bosch, A., Pulido-Leboeuf, P., Molina-Sánchez, L., Vallejos, A., & Martin-Rosales, W. (2000). *Intensive agriculture, wetlands, quarries and water management. A case study (Campo de Dalias, SE Spain). Environmental Geology*, *40*(1), 163-168.

Rádiková, Ž., Tajtakova, M., Kočan, A., Trnovec, T., Šeböková, E., Klimeš, I., & Langer, P. (2008). *Possible effects of environmental nitrates and toxic organochlorines on human thyroid in highly polluted areas in Slovakia. Thyroid*, *18*(3), 353-362.

Ranganathan, S., & Karmarkar, M. G. (2006). *Estimation of iodine in salt fortified with iodine & iron. Indian Journal of Medical Research*, 123(4), 531.

S. Eskiocak • C. Dundar • T. Basoglu • S. Altaner 2005 *The effects of taking chronic nitrate by drinking water on thyroid functions and morphology Clin Exp Med* (2005) 5:66–71 DOI 10.1007/s10238-005-0068-1

Salvato, J. A., Nemerow, N. L., & Agardy, F. J. (2003). *Environmental engineering*. John Wiley & Sons.

Savci S (2012). *An agricultural pollutant: chemical fertilizer. Int. J. Environ. Sci.* Dev. 3(1):77-80.

Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, et al. 2010. The challenge of micropollutants in aquatic systems. *Science* 313:1072–77

Sebilo, M., Mayer, B., Nicolardot, B., Pinay, G., &Mariotti, A. (2013).*Long-term fate of nitrate fertilizer in agricultural soils.Proceedings of the National Academy of Sciences*, *110*(45), 18185-18189.

Shawel, D., Hagos, S., Lachat, C. K., Kimanya, M. E., & Kolsteren, P. (2010). *Post-production losses in iodine concentration of salt hamper the control of iodine deficiency disorders: a case study in northern Ethiopia. Journal of Health, Population and Nutrition*, 238-244.

Smolders, A. J., Lucassen, E. C., Bobbink, R., Roelofs, J. G., &Lamers, L. P. (2010). *How nitrate leaching from agricultural lands provokes phosphate eutrophication in groundwater fed wetlands: the sulphur bridge. Biogeochemistry*, *98*(1-3), 1-7.

Speijers, G. J. A., & Van den Brandt, P. A. (2003).*Nitrate and potential endogenous formation of N-nitrosocompounds.WHO Food Additives Series*, *50*.

Tajtáková, M., Semanová, Z., Tomková, Z., Szökeová, E., Majoroš, J., Rádiková, Ž., ... & Langer, P. (2006). *Increased thyroid volume and frequency of thyroid disorders signs in schoolchildren from nitrate polluted area. Chemosphere*, *62*(4), 559-564.

Takem, G. E., Chandrasekharam, D., Ayonghe, S. N., & Thambidurai, P. (2010). *Pollution characteristics of alluvial groundwater from springs and bore wells in semi-urban informal settlements of Douala, Cameroon, Western Africa. Environmental Earth Sciences*, 61(2), 287-298.

Tang, Y., Jiang, H., & Bryan, N. S. (2011). *Nitrite and nitrate: cardiovascular risk–benefit and metabolic effect*. *Current opinion in lipidology*, *22*(1), 11-15.

Tay, C. (2004). *Report on the Ground water Quality of Akatsi and Ketu Districts in the Volta Region of Ghana*. CSIR – WRI.

Tjandraatmadja, G., Pollard, C., Sheedy, C., & Gozukara, Y. (2010). *Sources of contaminants in domestic wastewater: nutrients and additional elements from household products. Water for a Healthy Country Flagship Report: CSIRO*.

Tomer, M. D., & Burkart, M. R. (2003). *Long-term effects of nitrogen fertilizer use on ground water nitrate in two small watersheds*. *Journal of Environmental Quality*, *32*(6), 2158-2171.

Tomer, M. D., Meek, D. W., Jaynes, D. B., & Hatfield, J. L. (2003*). Evaluation of nitrate nitrogen fluxes from a tile-drained watershed in central Iowa. Journal of Environmental Quality, 32*(2), 642-653.

Tonacchera, M., Pinchera, A., &Vitti, P. (2010).*Assessment of nodular goiter*.*Best Practice & Research Clinical Endocrinology & Metabolism*, *24*(1), 51-61.

Trapp, L., & Will, J. (2010). Acquired methemoglobinemia revisited. *Dental clinics of North America*, *54*(4), 665-675.

Turner, B. L. & Haygarth, P. M. 2002 *Phosphorus forms and concentrations in leachate under four grassland soil types. Soil Sci. Soc*. Am. J.64, 1090–1097

Turner, B.L., Papházy, M., Haygarth, P.M. & McKelvie, I.D. 2002. *Inositol phosphates in the environment. Philosophical Transactions of the Royal Society of London* B 357, 449–469.

Umar, H., Muallima, N., Adam, J. M., &Sanusi, H. (2010).*Hashimoto's thyroiditis following Graves' di*sease.*Acta Med Indones, 42*(1), 31-35.

van Maanen, J. M., van Dijk, A., Mulder, K., de Baets, M. H., Menheere, P. C., van der Heide, D., ... & Kleinjans, J. C. (1994). *Consumption of drinking water with high nitrate levels causes hypertrophy of the thyroid*. *Toxicology letters, 72*(1-3), 365-374.

Wang, Q. S., Sun, D. B., Hao, W. P., Li, Y. Z., Mei, X. R., & Zhang, Y. Q. (2012). Human activities and nitrogen in waters.*ActaEcologicaSinica*, *32*(4), 174-179.

Ward, M. H., Kilfoy, B. A., Weyer, P. J., Anderson, K. E., Folsom, A. R., & Cerhan, J. R. (2010). Nitrate intake and the risk of thyroid cancer and thyroid disease. *Epidemiology (Cambridge, Mass.)*, *21*(3), 389.

Warren Viessman, Jr. Mark J. Hammer, Elizabeth M. Perez, Paul A. Chadik, 2009. *Water Supply and Pollution Control, 8th Edition. Pearson Publishers*

Waszkowiak, K., & Szymandera-Buszka, K. (2008). *Effect of storage conditions on potassium iodide stability in iodised table salt and collagen preparations*. *International journal of food science & technology*, *43*(5), 895-899.

World Health Organization. (1998). *The World Health Report 1998: Life in the 21st century a vision for all. In The world health report 1998: life in the 21st century A vision for all. World Health Organization.*

World Health Organization. (2003). *Guidelines for safe recreational water environments: Coastal and fresh waters* (Vol. 1). World Health Organization.

World Health Organization.(2010). *World health statistics 2010*.World Health Organization.

World Health Organization. (2011). Guidelines for drinking-water quality. Geneva: world health organization.

Zaki, A., Chaoui, A. A., Talibi, A., Derouiche, A. F., Aboussaouira, T., Zarrouck, K., ... & Himmi, T. (2004). Impact of nitrate intake in drinking water on the thyroid gland activity in male rat. *Toxicology letters*, *147*(1), 27-33.

Zaki, A.; Ait Chaoui, A.; Talibi, A.; Derouiche, A.F.; Aboussaouria, T.; Zarrouck , K.; Chait, A. and Himmi, T. ,2004. " Impact of nitrate in take in drinking water on the thyroid gland activity in male rat." Toxicol. Lett., (147): 27 – 33.(14)

Zimmermann, M. B. (2008). *Methods to assess iron and iodine status*. *British Journal of Nutrition*, *99*(S3), S2-S9.

Zimmermann, M. B. (2013). *Iodine deficiency and excess in children: worldwide status in* 2013. *Endocrine Practice*, *19*(5), 839.

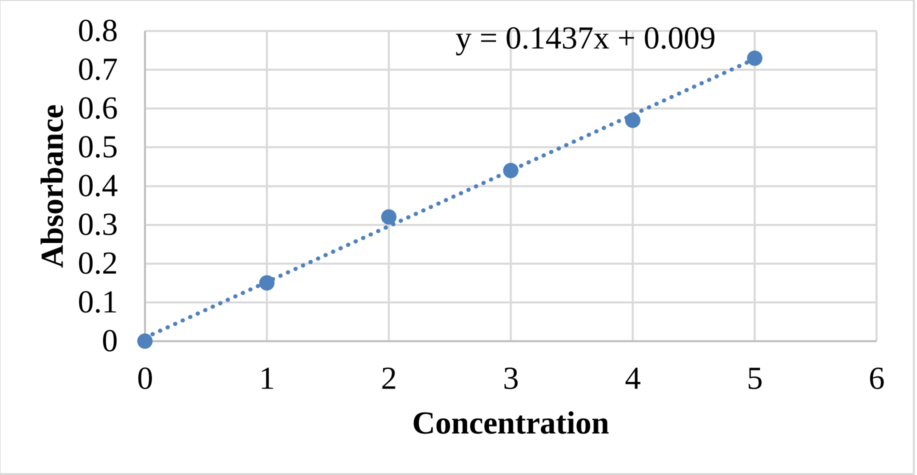
Zimmermann, M. B., &Andersson, M. (2012). *Update on iodine status worldwide. Current Opinion in Endocrinology, Diabetes and Obesity*, *19*(5), 382-387.

Zoeller, R. T. (2007). *Environmental chemicals impacting the thyroid: Targets and consequences.Thyroid* 17, 811–817.

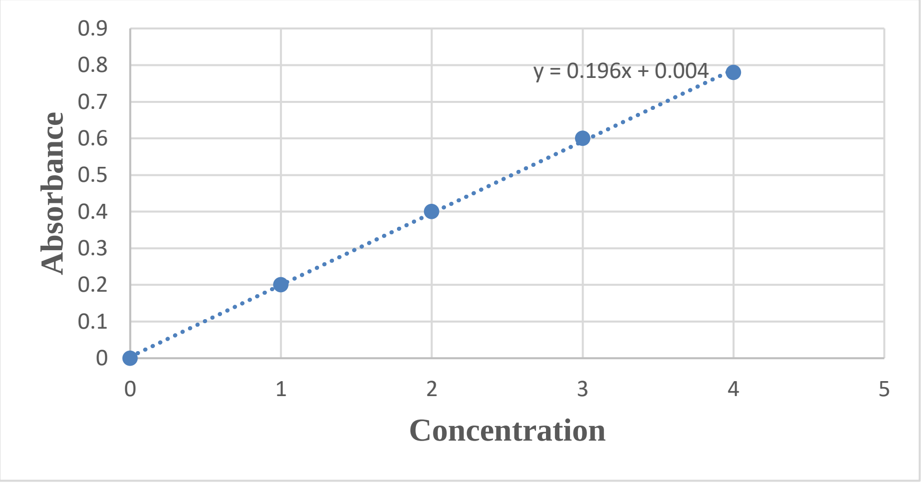
Zoeller, R. T. (2011). *Endocrine Disruption of the Thyroid and its Consequences in Development.* In *Multi-System Endocrine Disruption* (pp. 51-71). Springer Berlin Heidelberg.

APPENDICES

Appendix I: Standard curve for phosphate ions in drinking water



Appendix II: Standard curve for nitrate ions in drinking water



Appendix III: ANOVA table for phosphates ions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	52.156[a]	1	52.156	25.356	.000
Intercept	153.316	1	153.316	74.535	.000
Source	52.156	1	52.156	25.356	.000
Error	181.014	88	2.057		
Total	368.934	90			
Corrected Total	233.170	89			

a. R Squared = .224 (Adjusted R Squared = .215)

Appendix IV: ANOVA table for nitrates ions

	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11038272.095[a]	1	11038272.095	329.020	.000
Intercept	11139378.567	1	11139378.567	332.033	.000
Source	11038272.095	1	11038272.095	329.020	.000
Error	2918759.858	87	33548.964		
Total	27591372.674	89			
Corrected Total	13957031.954	88			

a. R Squared = .791 (Adjusted R Squared = .788)

Appendix V: ANOVA table for iodine ions

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	217452.092	1	217452.092	3.489	.203
	Error	124654.518	2	62327.259[a]		
Method	Hypothesis	124654.518	2	62327.259	141.648	.000
	Error	66002.173	150	440.014[b]		

a. MS(method)

b. MS(Error)

Appendix VI: Questionnaire

Dear Respondent,

My name is Sharon Tarus, a student at University of Eldoret. I am carrying out a study titled "**Relationship between inorganic components of environment, iodine levels in table salt and prevalence of goiter: A case study of Nandi Hills, Kenya".** The findings from this research will purely be used for academic purposes. Please fill in parts 1 and 2.

How old are you………………………………..

Gender of respondent: □ Male □ Female

PART ONE

1. Do you add table salt to your food?

□ Yes □ No

2. If yes, which type of table salt do you use?

□ Kensalt □ Kaysalt □ Bahari □ Others

3. At what stage of cooking do you add salt to food?

□ Early stage □ Mid stage □ Final stage □ on the table

4. Do you store salt in sealed containers?

□ Yes □ No

5. If no, please explain how you store your table salt---------------------------------------

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6. Is there any circumstance that you substitute salt?

□ Yes □ No

7. If yes please indicate the source --------------------------------------

PART TWO

1. Village name: ________________________________

2. What is the main source of water for general purposes at your home?

   a. River

   b. Stream

   c. Borehole

   d. Piped

   e. Rain

3. Which one of the two is the main source of drinking water?

   Borehole ☐ Stream ☐

4. Has any member of the household suffered goiter?

   Yes ☐ No ☐

5. How often do members of household suffer from goiter disorders ?

   Never☐ Rarely ☐ Often ☐Always ☐

Appendix VII: Map of the Study Area

(Source: Google maps)