



HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

SCHOOL OF POST GRAGUATE STUDIES

DEPARTEMENT OF CHEMISTRY

MASTER'S THESIS

ON

**DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS AND SELECTED
HEAVY METALS FOR DRINKING WATER SUITABILITY OF LAKE
ZENGENA,AWI ZONE,AMHARA REGION,ETHIOPIA**

BY:

ZEMENU KASSA ADELA

ADVISOR: Dr. SALAH HAMZA

NOVEMBER, 2024

HAWASSA, ETHIOPIA

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**A MASTER THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
OF HAWASSA UNIVERSITY, COLLEGE OF NATURAL AND COMPUTATIONAL
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REQUIREMENT FOR THE DEGREE OF MASTERS OF SCIENCE IN
CHEMISTRY**

NOVEMBER, 2024

HAWASSA, ETHIOPIA

Declaration

I, the undersigned, declare that this M.Sc. thesis entitled “**Determination of Physico-Chemical Parameters and Selected heavy metals for drinking water suitability of Lake Zengena, Awi Zone, Amhara Region, Ethiopia**” is my original work has not been presented for a degree or diploma of any other university and that all sources or materials for the thesis has been duly acknowledged.

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(Submission Sheet-1)

This is to certify that the thesis entitled “**Determination of Physico-Chemical Parameters and Selected heavy metals for drinking water suitability of Lake Zengena, Awi Zone, Amhara Region, Ethiopia**” submitted in partial fulfillment of requirements for the degree of Master of Science in Chemistry in Hawassa University. A record of original research carried out by **Zemenu Kassa Adela** (ID:-PGchemK/ 019/11), under my supervision, and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of thesis investigation have been duly acknowledged. Therefore, I recommended that it be accepted as fulfilling the thesis requirements.

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DEDICATION

The efforts of this research study work are dedicated to my beloved wife Bogalech Getaneh and all of my family members.

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ABBREVIATIONS AND CHEMICAL SYMBOLS

APHA	American Public Health Association
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DO	Dissolved oxygen
EBT	Eriochrome Black T'
EC	Electrical conductivity
EDTA	Ethylene di amine tetra acetic acid
EPA	Environmental Protection Agency
ESA	Ethiopian Standards Agency
FAAS	Flame Atomic Absorption Spectrophotometer
FAO	Food and Agriculture Organization
FEPA	Fair Employment Practice Act
IDL	Instrumental Detection Limit
MDL	Method Detection Limit
RSD	Relative standard deviation
SD	Standard deviation
TDS	Total dissolved solid
TSS	Total Suspended Solids
UNICEF	United Nations International Children's Emergency Fund
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

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Abstract

The determination of the physico-chemical parameters & heavy metals of any lake is important. The water quality test will identify whether the water is suitable for different purposes. This study aimed to determine the levels of some physico-chemical parameters and elements in Lake Zengena, Awi zone, Amhara regional state, Ethiopia using Atomic Absorption Spectrophotometer (AAS) & Palintest Photometer. For this study, six water samples were collected from two Lake Sites. Samples of the water from Lake Zengena were collected according to standard laboratory guideline for the analysis of water quality parameters & metals. The following water quality parameters were measured in the laboratory and in the field: temperature; pH; Electrical conductivity; TDS; turbidity, total hardness, dissolved oxygen, & BOD. There are two field sites and they were selected due to their differences in their position of the chance the contaminant can enter. The mean & standard deviation of the results obtained were - pH (6.82 ± 0.057) for site one & (6.62 ± 0.00816) for site two, electrical conductivity ($203.3 \pm 0.15 \mu\text{s/cm}$) for site one & ($199.1 \pm 0.471 \mu\text{s/cm}$) for site two, temperature (20.63 ± 0.057 °C) for site one & (20.13 ± 0.0416 °C) for site two. Total dissolved solids ($20.5 \pm 0.1 \text{ mg/l}$) for site one & (18.23 ± 0.124) for site two, turbidity ($0.52 \pm 0.015 \text{ NTU}$) for site one & ($0.41 \pm 0.00816 \text{ NTU}$) for site two, total hardness ($44.5 \pm 0.1 \text{ mg/l}$) for site one & ($41.4 \pm 0.081 \text{ mg/l}$). Dissolved oxygen (7.16 ± 0.11) for site one & (6.3 ± 0.0816) for site two, biological oxygen demand (22.46 ± 0.05) for site one & (20.3 ± 0.0816) for site two. In addition, chemical oxygen demand (104.56 ± 0.057) for site one and (102.16 ± 0.0471) for site two, alkalinity ($90.16 \pm 0.11 \text{ mg/l}$) for site one & (80.26 ± 0.124) for site two. In addition, the results of metals in water sample were as follow:-Pb, Cr & Ni were below detection limit. Ca (0.63 ± 0.02) for site one & (0.83 ± 0.016) for site two, Mg (0.31 ± 0.02) for site one & (0.43 ± 0.0081) for site two, Zn (1.65 ± 0.01) for site one & (1.85 ± 0.0081) for site two, K (5.8 ± 0.1) for site one & (4.66 ± 0.047) for site two. Fe (0.13 ± 0.02) for site one & (0.12 ± 0.0081) for site two, Cu (0.67 ± 0.015) for site one & (0.76 ± 0.0244) for site two. The statistical data analysis by using t-test shows that there was no significance difference between analyzed metals in site one and site two at ($p < 0.05$). The results of physico-chemical parameters & metals indicate that Lake Zengena is safe for the use of human being according to WHO.

Key words: - Zengena, Dissolved oxygen, Physico-chemical, heavy Metal, and Water

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

The physical and chemical properties of a lake in its natural state are shaped by various factors, including the surrounding topography, geological features, and inputs from rainfall, as well as interactions between water and rock, along with variations in climate change and heavy metals[1]. Lake systems are significantly affected by human activities within their watersheds leading to disturbances such as pollution and other forms of interference[2]. These disturbances create specific challenges that correlate with changes in various environmental factors assessing physico-chemical parameters and heavy metal concentrations in lakes is crucial for evaluating water quality, ecosystem health, and potential risks to human well-being. Lakes are essential for supporting biodiversity, offering recreational opportunities, and providing sources of drinking water. However, anthropogenic actions, including industrial discharges, agricultural runoff, urban development, and mining can greatly affect these freshwater ecosystems.

Lakes are integral components of the hydrological cycle, fulfilling multiple roles such as serving as habitats for aquatic organisms, regulating floods, and facilitating recreational activities. They function as natural reservoirs that support diverse ecosystems, contributing to regional biodiversity.

Water is one of the most vital and abundant substances in our ecosystem. All living organisms depend on water for survival and growth; it is believed that life itself originated in water. Currently, Earth is the only planet with about 70% of its surface covered in water. However, due to a growing human population, industrialization, and the excessive use of fertilizers in agriculture, water sources have becoming heavily polluted with harmful contaminants. Therefore, regular monitoring of drinking water quality is essential, as contaminated water can lead to a variety of waterborne diseases. Understanding biological processes is complex, but the chemistry of water provides valuable insights into ecosystem metabolism and highlights the general hydro-biological relationships at play[3]. Water is essential for human health and well-being, playing a crucial role in all aspects of life,

particularly in domestic and agricultural activities. Safe water is defined as affordable, readily available in sufficient quantities for drinking, food preparation, personal hygiene, and washing[4].

Water is vital for humans, animals, and plants; without it, life on Earth would not be possible. Humans require water not just for drinking, but also for a variety of other purposes, including bathing, washing, cooking, as well as for industrial, agricultural, and recreational activities[5]. Water is essential for life and development; without it, neither can thrive. A person's daily drinking water requirement typically amounts to about 7% of their body weight, highlighting its importance for healthy growth. However, using contaminated water tainted with harmful substances or pathogens, often due to poor sanitation can have detrimental effects on health [5].

Water supply sources largely depend on rainfall and include springs, rivers, lakes, and dug wells. As one of the most abundant substances in nature, water circulates through both the land and the human body, transporting nutrients while removing waste. Access to clean water is crucial for disease prevention and enhancing quality of life. While natural water sources contain various impurities that differ based on their origin, it's noteworthy that two-thirds of the Earth's surface is covered by water, and approximately 75% of the human body is composed of it[6]. Waste materials from activities such as mining or excavation significantly affect the quality of drinking water. Water-soluble substances that are spilled or stored on the land surface including: - manure, garbage and industrial wastes can either accumulate in mounds or disperse across the landscape. If these waste materials contain soluble substances, they pose a risk to nearby water sources[7]. The study highlights the severe impact of high pollution levels in rivers and groundwater, which can lead to various environmental consequences, including loss of biodiversity, increased water-related diseases, and decreased agricultural productivity. Mismanagement of water resources has significant socio-economic implications, making it essential to monitor physico-chemical parameters and conduct metal analyses to ensure water quality and the survival of aquatic life.

International standards, such as those set by the World Health Organization (WHO), provide guidelines for acceptable concentrations of various water quality parameters. However, these standards may vary between countries, emphasizing the need for localized assessments of water quality based on specific regional conditions and uses.

Heavy metals are a major concern in water pollution, stemming from industrial activities, agricultural runoff, and urban waste. Once released into the environment, these contaminants can persist for long periods due to their resistance to degradation. Metals like those that lead, mercury, cadmium, and arsenic tend to bio accumulate in organisms, particularly in critical tissues such as the liver and kidneys. This accumulation poses serious health risks to both wildlife and humans, as heavy metals can disrupt physiological functions and induce oxidative stress.

Understanding how heavy metals disperse and accumulate is vital for evaluating environmental risks and formulating effective remediation strategies. Currently, billions of people especially in developing regions, lack access to safe and adequate water[8]. Ensuring water purity is crucial for the well-being of populations worldwide, highlighting the urgent need for effective water management practices and pollution control measures.

1.2. Statement of the problem


Lake Zengena is one of the Crater Lakes, which provides economic, social, and ecological benefits to the local community. Water quality in lakes is a critical concern for ecological health, public safety, and sustainable resource management. Despite the essential role that lakes play in supporting biodiversity and providing resources for human use, many are increasingly threatened by pollution from various anthropogenic activities. Specifically, the presence of heavy metals and variations in physico-chemical parameters can significantly affect both aquatic ecosystems and the health of the communities that depend on these water bodies.

It plays a crucial role in supporting local ecosystems, agriculture, and community livelihoods. However, there is not enough information on physico-chemical parameters and heavy metals analysis in Lake Zengena. Understanding the physico-chemical parameters and heavy metals of the lake is essential for assessing its ecological health and sustainability. Despite its importance, there is limited data available on the current state of Lake Zengena's water quality. The available parameters of Lake Zengena are temperature, DO, turbidity, pH, EC, and nitrates. Key parameters such as temperature, pH, dissolved oxygen, turbidity, nutrient concentrations (e.g. nitrates, nitrites and phosphates), and TDS, hardness, alkalinity, electrical conductivity and others with those with heavy metal levels have not been systematically studied. This lack of information hinders effective management strategies and conservation efforts aimed at preserving the lake's ecosystem.

The aim of the current research is to address the critical knowledge gap concerning the physico-chemical properties and assessment of heavy metal contamination in Lake Zengena. Additionally, this study aims to determine systematically the physico-chemical parameters of Lake Zengena to provide a comprehensive assessment of its water quality. The findings will contribute to understanding the lake's health, inform local stakeholders, and guide future management practices to ensure the sustainability of this critical water resource. Therefore, the researcher was motivated to conduct the study for identification of the level of selected heavy metals and some physico-chemical parameters and to provide base line information about water quality of the lake Zengena. The main trigger to study this lake is to know its fitness for drinking purpose.





1.3. Objective of the Study

1.3.1. General Objectives of the Study

-  The general objective of the study was to determine physico-chemical characteristics and selected heavy metals in the lake Zengena.

1.3.2. Specific Objective of the study

The specific objective of the study was

-  To determine the drinking water physico-chemical parameters (temperature, pH, alkalinity, electrical conductivity, TDS, turbidity, hardness, DO, carbonate, bicarbonate, BOD, COD, fluorides, nitrates, phosphates , nitrites) and heavy metals Pb, Cr, Zn, and Ni of the lake.
-  To compare drinking water physico-chemical parameters of the lake with the WHO.
-  To evaluate the potential sources of pollution from the surrounding by agricultural activities and their impact on the lake's ecosystem and local communities.
-  To recommend the relevant body for the proper management of Lake Zengena.

1.4. Significance of the study

The study is mainly centered on the determination of physico-chemical parameters and heavy metals from water sources obtained from Lake Zengena. Water is the most important chemical substance for life and this water may be contaminated by different factors. Hence, assessing the quality of water from a scientific point of view is made to suggest information

about the levels of heavy metal ions and physico-chemical parameters according to guideline values of WHO.

The significances of the study will be used as input or baseline for further study and to motivate other researchers putting this as the stepping-stone for researchers who are interested in doing large-scale research on this lake. In addition, it may help societies and concerned bodies to have awareness of the ecosystem of the Lake by knowing necessary information about the lake from the study. Moreover, it brings about valuable information about the levels of heavy metals and physico-chemical parameters in the lake water that helps to take appropriate remedial action.

Generally, it can be used for potentially water quality assessment, identification of pollution sources, impact on human health, ecological significance, water management decisions, biodiversity conservation, environmental policy development, climate change research.

Therefore, some potential research questions that can be answered by this study are-

What are the current levels of physico-chemical parameters and heavy metals in Lake Zengena?

- ✎ What are the sources of pollution in the lake?
- ✎ How do the levels of heavy metals and other pollutants in the lake compare to international or national standards?
- ✎ What are the potential health risks associated with lake water use?
- ✎ How can the water quality of Lake Zengena be improved or conserved?

By answering above questions and others, the study can contribute to the development of effective strategies for water quality management, conservation and sustainable use of Lake Zengena.

1.5. Scope of the Study

The scope of the study was limited to the determination of physico-chemical parameters and selected heavy metals in Lake Zengena and further on comparing the study results with WHO standard.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. General Over View

Water is the second most essential requirement for life, following air. Consequently, the topic of water quality has been thoroughly explored in scientific literature. The most commonly accepted definition of water quality refers to the physical, chemical, and biological characteristics of water. It serves as an assessment of water's condition in relation to the needs of various living organisms and/or specific human uses or purposes[9].

The quality of water we utilize for various purposes in life can be assessed through physico-chemical parameter[7]. There are three primary types of water sources: rainwater, surface water, and seawater. Rainwater is the purest form of water, although it can contain dissolved gases such as oxygen, carbon dioxide, sulfur dioxide, and sulfur trioxide. Surface water is sourced from rivers and lakes and typically contains minerals like calcium, magnesium, sodium, potassium, sulfate, and chloride. Seawater, on the other hand, is predominantly salty and contains various elements in trace amount[6]. Water is an essential resource for all forms of life. Water quality refers to the composition of water and how its characteristics influence its usability. Assessing the quality of drinking water, particularly through the identification of heavy metals, is crucial due to its significant implications for human health. The term "water quality" encompasses the suitability of water for various uses and processes. A range of factors that may restrict its usability can define it.

2.2. Physico-chemical Parameters of water quality

The term "physico-chemical parameter (quality)" refers to the characteristics of water, including aspects like color and taste. These parameters can lead to toxic reactions, unexpected physiological responses such as laxative effects, and other undesirable effects during normal use[10].

Testing water before its use for drinking, domestic, agricultural, or industrial purposes is crucial. The selection of physico-chemical parameters for testing depends on the intended use and the required quality and purity of the water. Water can contain various impurities, including floating, dissolved, suspended, microbiological, and bacteriological contaminants.

Physical tests are essential for assessing the water's appearance and include measurements of temperature, color, odor, taste, pH, turbidity, and total dissolved solids (TDS). Chemical tests should evaluate biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen, alkalinity, hardness, acidity, chloride, fluoride, Nitrogen, Iron and manganese toxic inorganic substances, toxic organic substances, radioactive substances, and other characteristics. To ensure high quality and purity, water should also be tested for trace metals, heavy metals, and organic contaminants such as pesticide residues.

Drinking water must meet all these criteria and contain appropriate mineral levels. In developed countries, these standards are strictly monitored. Due to the low concentrations of heavy metals and organic pesticides in water, advanced analytical instruments and well-trained personnel are required for accurate testing.

Regular monitoring of water quality involves assessing various physico-chemical parameters. Physical parameters, which can be detected through sight, touch, and taste, include color, total dissolved solids, total suspended solids, electrical conductivity, temperature, and pH, Chemical parameters encompass alkalinity, hardness, and free carbon dioxide levels[10].

2.2.1. Temperature

Temperature significantly influences the metabolic activities of all living organisms and is a crucial factor in assessing water quality. It affects various parameters and plays a vital role in the solubility of gases in water. Changes in temperature can alter the physical and chemical properties of water, affecting the behavior of aquatic organisms, which may seek warmer or cooler areas after feeding. Generally, cooler water is more palatable than warmer water, and temperature can affect the acceptability of various inorganic constituents and chemical contaminants that influence taste. Elevated water temperatures can promote the growth of microorganisms, leading to potential issues with taste, odor, color, and corrosion[11]. While temperature is not directly used as a parameter for evaluating potable wastewater, it is a critical factor in natural surface water systems. The temperature of surface water significantly influences the types of biological species present and their activity levels. Additionally, it affects most chemical reactions occurring in natural water systems and the solubility of gases in the water[12].

2.2.2. pH

pH is a crucial parameter in assessing water quality, defined as the negative logarithm of hydrogen ion concentration. Measuring pH is one of the most commonly performed tests in water chemistry. The pH value indicates whether a water sample is acidic or alkaline. As pH increases, alkalinity rises, while a decrease in pH signifies increased acidity. Water that contains alkaline substances will have a higher concentration of hydroxide ions (OH^-), whereas acidic water will have more hydrogen ions (H^+). The pH scale typically ranges from 0-14 at 25°C. It is essential to maintain the pH within a range that is favorable for the specific organisms involved[13]. A pH value between 6 and 9 is crucial for aquatic life, as it helps protect freshwater fish and bottom-dwelling invertebrates. While a pH greater than 9 can allow fish eggs to hatch, it often results in the production of deformed young. For drinking water, a neutral pH of 7 is ideal. Values below 7 indicate acidity, meaning there are more hydrogen ions (H^+) than hydroxide ions (OH^-), making the water unsuitable for consumption. Water with a low pH can be toxic, while high pH levels often give the water a bitter taste. The pH scale is a dimensionless number that indicates the strength of an acidic or basic solution, serving as a measure of how acidic or basic the water is.

2.2.3. Alkalinity

Alkalinity refers to the acid-neutralizing capacity of water, encompassing the total of all titratable bases. It represents water's ability to accept hydrogen ions (H^+) and plays a crucial role in the biological treatment of natural water. Understanding the alkalinity of water is essential for determining the appropriate amount of chemicals needed for treatment.

Water with high alkalinity typically has a high pH and often contains elevated levels of dissolved solids. This can be detrimental for applications such as boilers, food processing, and municipal water systems. Alkalinity acts as a buffer and serves as a reservoir for inorganic carbon, influencing the capacity of water to support algae growth and aquatic life.

Essentially, alkalinity measures the water's ability to neutralize acidity and indicates the presence of bicarbonates and carbonates, along with higher concentrations of hydroxide (OH^-) ions. Additionally, it helps make water less susceptible to the effects of acid rain. However, if alkalinity levels are excessively high, the water may not be suitable for drinking, as this often correlates with a high pH and increased salt content, which can negatively affect plant growth[14] .

2.2.4. Total Dissolved Solid (TDS)

Water has ability to dissolve wide range of inorganic minerals or salts such as potassium, calcium, sodium, bicarbonates, chlorides, magnesium and others. These minerals produce unwanted taste. High concentrations of total suspended solids (TSS) can lead to numerous environmental issues. For instance, water bodies with elevated TSS levels often struggle to support diverse aquatic life. These suspended particles absorb heat from sunlight, raising water temperatures and reducing dissolved oxygen levels, which negatively influences aquatic organisms. TSS is a crucial parameter for assessing water quality for aquatic life, irrigation, and various domestic uses. When TSS is present in irrigation water, it is essential that it be not contaminated with hazardous chemicals from industrial sewage to ensure safety and effectiveness[15].

2.2.5. Electrical Conductivity

Electrical conductivity measures a water body's ability to conduct electrical current, which is directly related to its temperature and the concentration of dissolved minerals[16]. It serves as an indicator of the total ionized constituents in water, reflecting the sum of cations and anions present. As the concentration of ions increases, so does the conductivity, making it a key parameter for assessing water suitability for irrigation and firefighting?

Conductivity provides an indirect measure of various inorganic dissolved solids, such as nitrates, sulfates, and phosphates from calcium, magnesium, and other sources. While these dissolved solids are essential for aquatic life, excessively high concentrations can lead to water quality issues that negatively affect aquatic organisms[17].

2.2.6. Turbidity

Turbidity refers to the cloudiness of water and measures how well light can pass through it[18]. Suspended materials such as clay, silt, organic matter, plankton, and other particulate substances cause this cloudiness. Turbidity is quantified using a turbidity meter, which expresses the measurement in Nephelometric Turbidity Units (NTU). One NTU is equivalent to a 1 mg/L suspension of silica. Generally, turbidity levels above 5 NTU become noticeable to the average observer, while extremely muddy water can exceed 100 NTU. Groundwater typically has very low turbidity due to the natural filtration process that occurs as water seeps through soil[19].The impact of turbidity, it can increase the cost of water treatment for various applications[10]. Suspended particles can provide a refuge for harmful

microorganisms, shielding them from disinfection processes[9]. These particles serve as adsorption media for heavy metals such as mercury, chromium, lead, and cadmium, as well as hazardous organic pollutants like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and various pesticides[20]. Higher turbidity can reduce the availability of food in aquatic environments because suspended particles absorb more sunlight, raising water temperatures[21]. Consequently, this leads to a decrease in dissolved oxygen (DO) concentration, as warmer water holds less oxygen than cooler water.

2.2.7. Hardness

Water hardness refers to the soap-destroying capacity of water, primarily due to the presence of calcium and magnesium salts. It is a measure of the concentration of polyvalent cations (ions with a charge greater than one) in water, typically expressed as calcium carbonate (CaCO_3). In addition to calcium and magnesium ions, other ions such as bicarbonates, sulfates, chlorides, and nitrates may also be present. While iron (Fe^{2+}) and manganese (Mn^{2+}) can contribute to water hardness, they are usually found in much lower concentrations, especially in surface water.

Water hardness can be classified into two categories: temporary and permanent hardness. Temporary hardness arises from the presence of calcium and magnesium carbonates and bicarbonates, which can be removed by boiling. In contrast, calcium and magnesium chlorides, sulfates, or nitrates cause permanent hardness, which cannot be eliminated through boiling.

Water with high hardness values is considered hard, while water with low hardness values is classified as soft. Hard water requires more soap for effective cleaning because calcium and magnesium ions form complexes with soap, rendering it less effective. The hardness of water is a significant factor in industrial applications, as it can lead to scaling in heat exchange equipment, boilers, and pipes. Additionally, very hard water is generally unsuitable for drinking and irrigation due to its high salt content[20]. Generally, groundwater is harder than surface water.

Table 1:- Hardness description of water

Hardness range (mg/L as CaCO ₃)	Description of hardness
0-50	Soft
50-100	Moderately soft
100-150	Slightly hard
150-200	Moderately hard
200-300	Hard
>300	Very hard

2.2.8. Dissolved Oxygen (DO)

Oxygen is the single most important gas for most aquatic organisms; free oxygen (O₂) is used for respiration. Dissolved oxygen (DO) is a crucial parameter that provides both direct and indirect insights into the health of a water body. It is linked to various factors such as bacterial activity, photosynthesis, nutrient availability, and stratification[18]. As summer progresses, dissolved oxygen levels decline due to rising temperatures and heightened microbial activity[22]. DO levels below 1ppm will not support fish; levels of 5 to 6 ppm usually required for most of fish population. The elevated dissolved oxygen (DO) levels in summer can be attributed to higher temperatures and prolonged periods of bright sunlight, which affect the solubility of gases like O₂ and CO₂. During the summer months, the extended daylight and intense sunlight enhance photosynthesis in phytoplankton, which absorb CO₂ and release oxygen. This process likely explains the increased concentrations of O₂ observed during this season[10]. Dissolved oxygen (DO) in the sample can be measured titrimetrically using Winkler's method after a 5-day incubation period at 293 K. The difference between the initial and final DO levels indicates the amount of oxygen consumed by bacteria during this time. This procedure requires specialized BOD bottles that prevent atmospheric oxygen from entering the internal environment or atmospheric oxygen.

2.2.9. Carbonate (CO_3^{2-})

When the pH reaches 8.3, it indicates the presence of carbonates. This can be measured through titration with standardized hydrochloric acid, using phenolphthalein as an indicator[10] or by using Palintest photometer method. Below a pH of 8.3, carbonates are converted into an equivalent amount of bicarbonates. Titration can also be performed using pH meters or potentiometric methods or it can be also measured by photometric methods.

2.2.10. Bicarbonates (HCO_3^-)

Bicarbonates can be also measured through titration with standardized hydrochloric acid, using methyl orange as an indicator or by using Palintest method. Methyl orange turns yellow below a pH of 4.0. At this pH, carbonic acid decomposes into carbon dioxide and water[10]. Bicarbonates can be determined by using photometric methods.

2.2.11. Biochemical oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) quantifies the level of organic material contamination in water, expressed in mg/L[10]. It represents the amount of dissolved oxygen needed for the biochemical breakdown of organic compounds and the oxidation of specific inorganic substances, such as iron and sulfites. Unpolluted natural waters will have BOD of 5mg/L or less. BOD directly affects dissolved oxygen in rivers and streams. The BOD test is usually performed over a five-day period and temperature 20°C as per global standard[22]. BOD is function of time. At time, zero no oxygen will be consumed then BOD is zero.

2.2.12. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) measures the overall magnitude of oxidizable materials present in a sample. It is another metric for assessing organic contamination in water, expressed in mg/L. It measures the amount of dissolved oxygen needed for the chemical oxidation of organic materials present in the water. Both BOD (Biochemical Oxygen Demand) and COD serve as important indicators of the environmental health of surface water sources. While they are frequently used in wastewater treatment, they are less commonly applied in general water treatment processes[22]. COD is always greater than BOD.

2.3. Some Anions found in Water

2.3.1. Fluorides

Fluorine is a common element that does not exist in its elemental form in nature due to its high reactivity. Instead, it is widely distributed in the environment as fluorides, found in the air, soil,

rocks, and water. Many water sources contain trace amounts of fluorides, with higher concentrations often linked to underground sources. In regions rich in fluoride-containing minerals, well water can have fluoride levels reaching up to 10 mg per litre. Additionally, fluorides can enter rivers through industrial discharges, agricultural practices, and pharmaceuticals[22]. In groundwater, fluoride concentrations vary depending on the type of rock the water passes through, but they typically do not exceed 10 mg/L[23]. Most of the fluoride that people consume comes from fluoridated water, food, toothpaste, and other dental products[24]. The digestive system absorbs about 80% or more of fluoride consumed orally[25]. In adults, about 50% of absorbed fluoride is retained, and bones and teeth store about 99% of fluoride in the body the other 50% is excreted in the urine [26]. In young children, up to 80% of absorbed fluoride is retained, because bones and teeth take more than adults do. Fluoride absorption in bones and teeth decreases with increasing age[27].

Both fluoride deficiency and excess can negatively affect human health. Extensive research has been conducted on the effects of high fluoride levels in drinking water, which can lead to skeletal problems in bones and teeth, particularly in children and pregnant women[28]. Lower limit of fluoride content is not specified, fluoride content of 0.5 mg/L will increase the risk of dental caries, affects the formation of dental enamel, as well as decrease the mineralization of bones, especially for children who are in the body growth stage[29]. WHO recommends the fluoride concentrations in drinking water which range from 0.5 to 1.5 mg/L for tooth deterioration prevention[29]. Since low and high contents of fluoride in drinking water both pose threats on human health , it is of great importance to evaluate the fluoride content in drinking water sources accurately[30].

2.3.2. Nitrates

Almost every rainwater and ground water aquifers have a little nitrate-nitrogen. Nitrates are commonly present in natural, drinking and wastewaters. High concentrations of nitrate in domestic water supplies can pose significant health risks to humans. Nitrate is an important control test for water supplies. Nitrate is found naturally in the environment and is an important plant nutrient. It is present at varying concentrations in all plants and is a part of the nitrogen cycle[31]. Drinking waters containing excessive amounts of nitrates can cause methaemoglobinaemia in bottle-fed infants (blue babies). Nitrate primarily originates from inorganic fertilizers and from breakdown of natural vegetation. In the soil, these fertilizers, along with organic nitrogen from waste, undergo decomposition to produce ammonia, which is

then converted into nitrite and nitrate. Plants absorb nitrate during their growth, utilizing it to synthesize organic nitrogenous compounds. However, excess nitrate can easily leach into groundwater and enter aquatic systems through various pathways, including overland flow, surface runoff, and groundwater discharge during precipitation events. Precipitation acts as a key driver for this transport by contributing to soil erosion. Elevated nitrate levels in surface or groundwater typically indicate contamination from agricultural sources, such as fertilizers and manure runoff[32]. Ground water is preferred as a source of potable water because it is available throughout the year and less contaminated than surface water[33]. However, according to studies in both developed and developing countries, nitrate levels in groundwater have been increasing and can present serious problems[34] .

A rapid assessment of drinking water quality in Ethiopia revealed that 32% of the wells tested were contaminated with nitrate[6]. The increased application of fertilizers on farms is driven by the loss of organic matter in deteriorating soils and the urgent need to produce more food for a rapidly growing population[35]. Despite the extensive use of fertilizers, the quality of surficial groundwater used for drinking water in Ethiopia remains a concern. High levels of nitrate in drinking water pose significant health risks, including thyroid gland dysfunction, gastric cancer, and methemoglobinemia an oxygen transport issue in infants under six months [36]. Additionally, elevated nitrate levels can lead to health complications for pregnant women[37]. Both the World Health Organization (WHO) and Ethiopian authorities have established a guideline value of 50 mg/L for nitrate in drinking water[38].

2.3.3. Phosphates

Phosphate is a chemical compound that contains phosphorus, an essential nutrient for plants and often a growth-limiting factor. In the human body, phosphorus plays a crucial role in building and repairing bones and teeth, supporting nerve function, and facilitating muscle contractions. Approximately 85% of the phosphorus in phosphate is stored in bones, while the remainder is found in tissues throughout the bloodstream and soft tissues[39]. In natural water, phosphorus typically exists in dissolved form as orthophosphate, which is the most significant form of phosphorus present[40]. Orthophosphate is the most thermodynamically stable form of phosphate and is the form commonly identified in the laboratory analysis[16]. Phosphate enters the surface of water and ground water through natural sources and anthropogenic sources. Natural sources of phosphate in both surface and ground water include atmospheric deposition, natural decomposition of rocks and minerals, weathering of solid inorganic materials, decaying

biomass, runoff, and sedimentation. Anthropogenic sources include; fertilizers, wastewater, and septic system effluent, animal wastes, detergents industrial discharge, phosphate mining, drinking water treatment forest fires, synthetic material development surface[41]. High concentration of phosphate in water bodies is an indication of pollution and largely responsible for abnormal growth of algae[42]. Since phosphorus is tightly bounded by soil particles, and thus moves into surface water bodies from runoff, and it migrates with ground water flows, since ground water discharges into surface water. An excess of phosphate enters the water body and choke up the waterway. Phosphate is nontoxic unless they are present in very high levels. Digestive problems such as: - sever constipation, nausea vomiting, and diarrhea could occur from extremely high levels of phosphate[34,43]. Moreover, algal blooms have been associated with health issues, including skin irritation and even fatalities in both humans and animals, depending on the type of algae and the duration of exposure[32,44,45].

2.3.4. Nitrites

Nitrites are found in natural waters as an intermediate product in the nitrogen cycle. Nitrite is harmful to fish and other forms of aquatic life and the nitrite level must be carefully controlled in water used for fish farms and aquariums[46–48]. The nitrite test is also applied for pollution control in wastewaters, and for the monitoring of drinking water[46].

The Palintest Nitricol test provides a simple method of measuring nitrite. Nitrogen levels over the range 0 - 0.5 mg/L N. Higher levels can be determined by diluting the sample.

2.4. Some Important metals and heavy Metals

When toxic metals enter the body, various processes can occur. Heavy metals often bind to thiol groups, lipids, proteins, and nucleic acids. This binding frequently takes place through thiol (-SH) groups, leading to modifications of cysteine residues in proteins, which can result in protein inactivation. Such alterations can disrupt the intercellular redox state, contributing to an imbalance in antioxidant defenses and potentially leading to liver injury [9]. The pollution of aquatic environments with heavy metals has become a global issue in recent years, as these metals are indestructible and many exhibit toxic effects on humans[49].

The term "heavy metal" typically refers to elements with densities greater than 5.0 g/cm³[50]. Therefore, these metals are found in trace amounts in the Earth's crust but have increased significantly due to human activities, such as industrial processes and the use of metals, alloys, and metallic compounds. This anthropogenic impact raises their natural

background levels, allowing them to enter the human body primarily through two routes: inhalation and contaminated water consumption, as well as indirect exposure through food consumption.

Certain heavy metals pose significant risks due to their toxic effects on humans, plants, and animals, and their rising prevalence in the environment. Toxicity occurs when these metals are ingested above recommended limits[51,52]. While each metal may present unique toxicity symptoms, common signs associated with poisoning from cadmium, lead, arsenic, mercury, zinc, copper, and aluminum include gastrointestinal disorders, diarrhea, steatosis, tumors, hemoglobinuria, paralysis, vomiting, depression, and pneumonia. The nature of these effects can be toxic (acute, chronic, or sub-chronic), neurotoxic, carcinogenic, or mutagenic[53–55]. Rapid industrialization, population growth, and the indiscriminate use of natural resources have significantly exacerbated water pollution issues. The mechanisms through which heavy metals induce carcinogenicity remain unclear and complex. It is believed that these metals interact with regulatory proteins involved in cell cycle regulation, DNA synthesis and repair, and apoptosis[56]. When exposure to heavy metals leads to resistance against apoptosis, it undermines a fundamental cellular defense mechanism[57,58].

2.4.1. Lead (Pb)

Lead (Pb) is widely present in the environment, found in air, food, water, and soil. It ranks second on the Environmental Protection Agency's "Top Hazardous Substance Priority List." With the atomic number 82, lead is a main group element in the carbon group, derived from the Latin word "plumbum". In nature, it rarely occurs as a free metal and is primarily found in the form of its main ore, galena (PbS). Lead commonly exists in two oxidation states: Pb^{2+} and Pb^{4+} , with the divalent form being more stable in most aquatic environments.

The concentration of lead in surface and groundwater varies based on pollution sources. Lead in water can originate from various waste materials, including batteries, ammunition, metal products (such as sheet metal, solder, & pipes), medical equipment used for radiation protection and surgical instruments, as well as paints, ceramics, and scientific equipment[59–61].

Everyone is exposed to trace amounts of lead through air, soil, household dust, food, drinking water, and various consumer products. Inorganic lead, primarily in the +2 oxidation state, is released into water from numerous industrial and mining activities, including pigments, anticorrosion coatings, lead smelting, alloys, and barriers[58,62–64]. The use of lead is in the

pipes drains, and soldering materials for many years. Millions of homes built before 1940 still contain lead (e.g. in painted surfaces), leading to chronic exposure from weathering, flaking and dust. Every year industry produces about 2.5 million tons of lead throughout the world. Most of this lead is used for batteries. The remainder is used for cable coverings, plumping, and fuel additives. Other uses are as paint pigments in PVC plastics, X-ray shielding crystal glass production, and pesticides. Natural sources of lead are wind-blown dust, weathering of metal bearing rock and soil. Anthropogenic sources are Pb batteries, paint pigments, coating pesticides, PVC plastics[65].

The health effects of lead are birth defects, mental retardation, allergies shaky hands ,muscular weakness, paralysis, anemia, increased blood pressure ,kidney damage ,decreased IQ in children ,decreased sperm motility,(movement) and pregnancy disorders[65] and it can also induce neurological ,respiratory and urinary disorder due to inflammatory mechanisms[66]. It is highly toxic which has adverse effects on the neurological biological and cognitive functions in the bodies. Lead is commutative poison and a possible human carcinogen[55]. In addition, lead causes the development of autoimmunity in which a person's immune system attacks its own cells. This can lead to joint disease and ailment of kidneys, circulatory system and neurons. At higher concentrations, lead can cause irreversible brain damage[52].

The target organs of lead are bones brain, blood, kidney, and thyroid glands. Lead is toxic metal with no metabolic benefits to human and aquatic biota. It can cause permanent damage to the health of both children and adults. Adults and children who drink water-containing Pb in excess could experience kidney problems or high blood pressure and delays in their physical or mental development respectively[67]. Environmental lead is bound particle with low mobility and bioavailability. Lead is not an essential substance for living things. Lead can cause biological effects that can vary with dose and duration of exposure. The impact can only be in the form of enzyme inhibition that causes morphological changes to the most severe in the form of death. Children are more susceptible to the adverse effects of lead than adults[68].

2.4.2. Zinc (Zn)

Zinc is an essential trace element found in virtually in all food and potable water in the form of salts or organic complexes. The diet is usually the principal source of zinc. The level of zinc in a ground water and surface water normally don't exceed 0.01 and 0.05mg/L respectively, concentrations in tap water can be much higher as a result of dissolution of Zn

from pipes. It was considered that the derivation of a formal guideline value for Zn is not required. However, drinking water containing Zn at the levels above 3mg/L may not be acceptable to consumers and it may affect the acceptability of drinking water. So, no health-based guideline value has been proposed for zinc in drinking water since it is not of health concern at levels found in drinking water. Zinc is ranked as 75th in the Environmental Protection Agency "Top Hazardous Substances Priority List". Zinc or spelter (which may also refer to zinc alloys), is a metallic element with the symbol Zn and atomic number 30. Zinc is 24th the most abundant element in the earth's crust and has five stable isotopes. The most common zinc ore is zinc blende (ZnS) mineral. Zinc is the essential trace element necessary for plants, animals and microorganisms. Corrosion-resistant zinc plating of steel (hot-dip galvanizing) is the major application of zinc. Other applications are in batteries and alloys, such as brass. Zinc, which is found naturally in water from industrial wastes, metal plating and plumbing, is an essential nutrient in the diet of man because it acts as a catalytic or structural component in many enzymes that are involved in energy metabolism and in transcription and translation of RNA, cell division, cell activation, connective tissue growth, maintenance, development, and homeostasis. It is a trace metal, meaning that the body only needs small amounts, and yet it is necessary for almost 100 enzymes to carry out vital chemical reactions. It is a major player in the creation of DNA, growth of cells, building protein, healing damaged tissue, supporting a healthy immune system, proper thyroid function, blood clotting, cognitive functions, fetal growth, regulates body fluid pH and sperm production.

It plays an important role in determining the outcome of pregnancy [59]. Supporting neurobehavioral development on a positive note and found in all body tissues and secretions in relatively high concentrations, with 85% of the whole body zinc in muscle and bones, 11% in the skin and liver, and the remaining in the other all tissues, with the highest concentrations in the prostate and parts of the eye [66, 67]. Natural sources of zinc are volcanic emission, biogenic emission, and sea salt sprays. Anthropogenic sources are Mining Metallurgical operations, fertilizers, electroplating, smelting and ore processes. The health effects of zinc at very high concentrations are abdominal discomfort, headache, and respiratory complications, and flu-like symptoms, shortness of breath [70]. Zn compounds are corrosive and irritating to the skin, eye, mucous membrane and digestive tract causing nausea, vomiting and special types of dermatitis known as "zinc box" [71].

2.4.3. Nickel (Ni)

Nickel (Ni) is the 24th most abundant element in the Earth's crust, comprising about 3% of the composition of the earth. It is the 5th most abundant element by weight after iron, oxygen magnesium and silicon. It is the member of the transition series and belongs to group VIII B of the periodic table. It has a symbol Ni and atomic number 28. Nickel is a naturally occurring element that can exist in various mineral forms. Natural nickel is mixture of 5 stable isotopes. The prevalent oxidation states under environmental conditions is Ni (II), nickel in the +2 – valence state .Other valences (-1, +1, +3 and +4) are also encountered, though less frequently[64,72,73]. Nickel is nutritionally essential trace metal for at least several animal species, microorganisms and plants, and therefore either deficiency or toxicity symptoms can occur when, respectively, too little or too much Ni is taken.

Nickel and Nickel compounds have many industrial and commercial applications. Most Nickel is used for the production of stainless steel and other nickel alloys with high corrosion and temperature resistance. Nickel metal and its alloys are used widely in the metallurgical, chemical and food processing industries, especially as catalysts and pigments.

The nickel salts of greatest commercial importance are nickel chloride, sulphate, nitrate and carbonate hydroxide acetate and oxide[70–72] . Nickel is one of the many trace metals found widely distributed in the environment, being released from both natural sources and anthropogenic activity, with input from both stationary and mobile sources. It is present in the air, water, soil, and biological materiel. Natural sources of atmospheric nickel levels include wind-blown dust, derived from weathering of rocks and soils, volcanic emissions, forest fires and vegetation. Environmental sources of lower levels of nickel include tobacco, dental or orthopedic implants, stainless steel kitchen utensils and inexpensive jeweler[73]. Nickel is normally present in human tissues and, under conditions of high exposure; these levels may increase significantly[73,75]. The absorption of nickel is dependent on its physicochemical form, with water -soluble forms (chloride, nitrate, sulphate) being more readily absorbed. Nickel is a ubiquitous metal frequently responsible for most common causes of allergic skin reactions[73]. Its hypersensitivity also causes asthma, conjunctivitis, inflammatory reactions. Nickel metal dusts and some nickel compounds are extremely potent carcinogens after inhalation but also that carcinogenic risk limited to conditions of occupational exposure[30,76]. Differences in the carcinogenic activities of nickel compounds may reflect variations in their capacities to provide nickel ions (e.g. Ni²⁺) at critical sites within target cells.

2.4.4. Copper (Cu)

Cu is both essential nutrient and a drinking water contaminant. Copper in drinking water supply usually arises from the corrosive action water leaching Cu from Cu pipes in buildings. High concentration can interfere with intended domestic use of the water.

Copper is been ranked as 25th in the Environmental Protection Agency “Top Hazardous Substance Priority List”. Copper is a member of transition elements with the symbol Cu (from Latin: cuprum) and atomic number 29. It is ductile metal with very high thermal and electrical conductivity. It is the next to silver in the electrical conductivity. Pure copper is soft and malleable; a freshly exposed surface has a reddish-orange color. The presence of the element copper in nature can be found in the form of free metals but it is more commonly found in compounds[70]. Cu is included in the essential metal group, where in low levels it is needed by organisms as coenzymes in the body’s metabolic processes, its toxicity only appears in high levels. Depending on the when concentration of 0.01-ppm phytoplankton will die because Cu inhibits enzyme activity in the phytoplankton cell division[30]. Cu concentration in the range of 0.15-0.2ppm in the water bodies will kill fish, copper content in waste and leachate can easily spread following the movement of water flow in the soil. Most copper widely used in the electrical industry to make heat and electrical conductor equipment’s such as electrical wire cable and motors. This is because Cu conducts both heat and electricity very well and can be drawn in to wires. It also has uses in construction (for example roofing and plumbing), industrial machinery (such as heat exchanger) and a constituent of various metal alloys. The metal and its alloys have been used for thousands of years. Its alloys for example are bronze i.e. an alloy of Cu and Sn) used to make coin, medal, bells and machinery parts. Another alloy is brass i.e. an alloy of Cu and Zn used to make hard ware tops, terminals and pipes. CuCl_2 , CuCO_3 and $\text{Cu}(\text{OH})_2$ used as pesticides.

Its compounds are commonly encountered as copper salts, which often impart blue or green colors minerals such as turquoise and have been widely used historically as pigments. At lower concentrations, it is an essential trace nutrient to all higher plant and animal life, whereas, insufficient amounts, they are poisonous to higher organisms. Copper is required for the proper functioning of many important enzyme systems. Copper-containing enzymes include ceruloplasmin, cytochrome-c oxidase, tyrosinase, monoamine oxidase, and lysyl oxidase and phenylalanine hydroxylase[77,78]. The natural sources of copper are naturally presents windblown dust and volcanic eruptions; anthropogenic sources are smelting plants,

electroplating, steel industry batteries. The environmental toxicity of copper is dental prostheses, asthma, respiratory cancer, heart disorders[70] . There is inadequate evidence to state whether or not Cu has the potential to cause cancer from a lifetime exposure in drinking water. Wilson diseases are a rare inherited disorder that causes too much copper toxicity. Wilson diseases generally appear in late childhood[9].

2.4.5. Chromium (Cr)

Chromium is the most abundant transition metal in the earth crust. It has a symbol Cr and an atomic number 24. Chromium is a grey, hard metal commonly found in trivalent state in nature. Hexavalent (Chromium VI) compounds are also found in very small quantities. Chromite (FeCr_2O_4) is the only ore containing a significant amount of Chromium. The ore has not been found in the pure form; its highest grade contains about 55% chromic oxide [79]. Chromium (VI) in a soil can be rapidly reduced to Chromium (III) by organic matter. As Chromium is almost ubiquitous in nature, chromium in the air can may originate from wind erosion of shales, clay and many other kinds of soil.

It is generally accepted that chromium is an essential element for humans[79]. Sodium chromate and dichromate are now among the most important chromium products, and are used chiefly for chromic acid, chromium pigments, in leather tanning and for corrosion control[80]. Nichrome wire, commonly used in chemistry laboratories for flame testes, is an alloy of Chromium and Nickel. Ferrochrome, iron chromium alloy, is used in the production of the stainless steel, bright, shiny steel that is strong and resistant to oxidation (rust). Stainless steel production consumes most of the chromium produced annually. Sodium and potassium chromates are used as pigments and corrosion inhibitors in the heating systems. Chromium is important in the breakdown of fat and carbohydrates.

It stimulates fatty acid and cholesterol synthesis, they are important for brain function and other body processes. Cr also aids in the insulin action and glucose breakdown. The bronchial tree is the primary target organ for carcinogenic effects of chromium (VI). Chromium is generally present in the trivalent or hexavalent oxidation states but may also occur in biological materials in the quadrivalent or pentavalent state. It has been suggested that Chromium concentration in the human lungs increases with age[79]. Absorption by inhalation exposure appears to occur rapidly for water-soluble Chromium (VI) compounds. Chromium (III) is the retained to greater extent in the lungs than is Chromium (VI)[66].

Chromates with low water solubility are mainly cleared to the gastrointestinal tract, whereas Chromates that are more soluble are absorbed into the blood[79]. Chromium transported by blood is distributed to other organs, the most significant retention being found in the spleen, liver and bone marrow[1,79]. The primary route of elimination is urinary excretion. Interaction has been demonstrated with smoking found higher level of chromium in the urine of smokers than for nonsmokers[81].

Toxicological effects Chrome ulcers, corrosive reactions on the nasal septum, acute irritative dermatitis and allergic eczematous dermatitis have been recorded among subjects exposed to chromium (VI) compounds[79]. Systematic effects of chromium compounds in the kidneys, liver, skin and airways of animal and humans have been reviewed[79,81–83] appearance of ulcerations and subsequent perforations of the nasal septum following exposure to chromium (VI) compounds is considered to be one of markers of toxic effects resulting from exposure[79,80,83,84]. Deposition of particulates containing chromium (VI) may explain the occurrence of septal ulceration at low levels of exposure[85]. Cr is emitted to the surrounding through leakage, bad storage or inappropriate waste disposal ways.

2.4.6. Calcium (Ca)

Calcium is an important or essential nutrient for plants and animals, essential for bone nervous system, and cell development. Recommended daily intakes for adults are between 800 to 1200mg/day. Most of this is obtained in food; drinking water typically accounts for 50-300mg/day, depending on the water hardness and assuming ingestion of 2L/day. Calcium in a food and in water is essentially nontoxic. A number of studies suggest that water hardness protects against cardiovascular disease. One possible adverse effect from ingestion of high concentration of calcium for long periods may be a greater risk of kidney stones. The presence of Ca in the water decreases the toxicity of metals to aquatic life. Thus, the presence of Ca in water is beneficial and no limits on Ca have been established for protection of human or aquatic health [20,65,86].

2.4.7. Iron (Fe)

Iron is the second-most abundant metal next to aluminum, in the earth's crust and it is the fourth most abundant element. Iron occurs in nature, it is found in many natural, and treated waters. Iron is an objectionable constituent in both domestic and industrial water supplies. The presence of iron affects the taste of beverages and causes unsightly staining of laundered clothes, plumping fittings, swimming pool surfaces and the like. It constitutes about 4.7% of

the earth's crust by weight. It is never found as a free metal in nature. It occurs in nature only in the form of compounds, such as oxides, carbonates and sulphides. Iron is a grey, lustrous, malleable and ductile metal. It is a good conductor of heat and electricity. It has a high melting point (1580°C) and a high density (7.87g/cm³). The chief ores of iron are hematite (Fe₂O₃), limonite (Fe₂O₃.H₂O), magnetite (Fe₃O₄), and siderite (FeCO₃). It can also be found in the form of iron pyrites (FeS₂). Fe is also one of the most abundant heavy metals found in the Earth's crust. Fe contains both +2 and +3 oxidation states [87]. Fe is found in natural fresh waters at levels ranging from 0.5 to 50 mg/L. Fe may also be present in drinking waters because of the use of Fe coagulants or the corrosion of steel and cast iron pipes during water distribution. Fe is an essential element in human nutrition, particularly in the Fe (II) oxidation state [88].

Fe may affect the acceptability of drinking water. However, it is not of health concern at levels causing acceptability problems in drinking water. Therefore, no health based guideline value for Fe in drinking water is proposed. The taste and appearance of drinking water can usually be affected at levels above 0.3 mg/L Fe [88]. The formation of insoluble iron deposits is troublesome in many industrial applications and in agricultural water uses such as drip feed irrigation. In industry, iron salts occur through corrosion of plant and equipment, and from industrial processes. Iron is therefore an important test for the monitoring of natural and drinking waters, for corrosion control in industry and for checking of effluents and wastewaters [88].

2.4.8. Magnesium (Mg)

Magnesium is an essential nutrient for plants and animals, essential for bone and cell development. Recommended daily intake for adults is 400-450mg/day of which drinking water can supply from 12 to 250 mg/day, depending on the Mg concentration and assuming ingestion of 2L/day.

In general, the presence of Mg in water is beneficial and no limits on the Mg have been established for protection for protection of human or aquatic health [89]. There are no primary or secondary drinking water standards for Mg. Mg in drinking water may provide nutritional benefits for persons with Mg deficient diets [90].

2.4.9. Potassium (K)

Potassium is an essential element in the humans and other living organisms [91]. Potassium occurs widely in the environment, including all natural waters. It can also occur in drinking water because of the use of KMnO_4 as an oxidant in the water treatment. In some countries, KCl is being used in ion exchange with Calcium and Magnesium ions. Possible replacement or partial replacement of sodium salts with potassium salts for conditioning desalinated water has been suggested. The later seems to be an unlikely development at this stage, in view of the cost difference. Currently, there is no evidence that levels in municipality treated drinking water, even water treated with KMnO_4 , are likely to pose any risk for the health of consumers. It is not considered necessary to establish a health based guidelines value established for K in drinking water[92].

2.5. Atomic Absorption Spectrophotometer (AAS)

AAS is an easy, high-throughput, and inexpensive technology used primarily to analyze elements in solution. So, AAS is used in food and beverage, water analysis, environmental analysis, mining, clinical research, and pharmaceutical analysis.

Atomic absorption spectrophotometer is an analytical technique that measures the concentration of elements. It is so sensitive that can measure down to parts per billion of a gram ($\mu\text{g dm}^{-3}$) in a sample. The technique makes use of wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another higher energy level. It is important instrumental technique for both quantitative and qualitative determination of chemical elements employing the absorption of optical radiation by free atoms in the gaseous states. This method measures the concentration of the element by passing light of a specific wavelength emitted by a radiation source of a particular element through clouds of atoms from a sample. AAS can be used a metal analysis suitable for the determination of approximately 70 elements[93]. This technique is applicable to most of gas phase elements over a wide range of concentrations and involves detecting, measuring and analyzing the radiation that is either absorbed or emitted from the atoms or from the ions of elements of interest. It involves three techniques: absorption, emission and fluorescence. In all of the above process, the sample is decomposed by intense heat into hot gases consisting of free atoms and ions of the element interest. As atoms are the simplest and purest forms of matter and cannot rotate or vibrate as a molecule does when subjected to high-energy radiation, electrons within the atom undergoes

transitions[94]. In AAS, the sample is atomized *i.e.* converted into ground state free atoms in the vapor state and a beam of electromagnetic radiation emitted from excited atoms is passed through the vaporized sample. Some of the radiation will be absorbed by the atoms in the sample. The greater the number of atoms there is in the vapor, the more radiation is absorbed. The amount of light absorbed is proportional to the number of those atoms. Calibration curve is constructed by running several samples of known concentration under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the concentration in the unknown sample. Consequently, an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed. The high energy is commonly produced by FAAS, GFAAS, ICP-OES, & XRF[95]. So, this belongs to major atomic spectroscopy namely absorption, emission and fluorescence.

2.5.1. Principle of Operation

AAS, technique makes use of atomic absorption spectrometry to assess the concentration of analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies on Beer Lambert Law. The electrons of the atoms in the atomizer can be promoted to higher orbitals for a short period by absorbing a defined quantity of energy. This amount of energy that is wavelength is specific to a particular electron transition in a particular element. The common source of light is a hollow cathode lamp[96]. This contains a tungsten anode and a cylindrical hollow cathode made of the element to be determined.

2.5.2. Analysis of metals and physico-chemical parameters

Mainly many analytical techniques are used to identify the metals in samples having the choice frequently based on the level of sensitivity and precision needed. Both macro and micro elements can be determined by various spectroscopic or chromatographic methods, such as atomic absorbance spectrometry by using flame (FAAS) or graphite furnace (GFAAS) atomization, atomic emission spectroscopy (AES), inductively coupled plasma mass spectrometry(ICPMS) , X-ray fluorescence(XRF) and ion chromatography(IC). In this study, metals in the samples of water sample were determined by using FAAS. Before analysis, all of these techniques including FAAS requires sample pretreatment.

CHAPTER THREE

3. MATERIALS AND METHOD

3.1. Description of Study Area

Zengena Lake, which is less known, is the highland Crater Lake located in the western Ethiopia, in the Awi zone of Amhara region, Ethiopia. Lake Zengena is an attractive lake situated in Kessa- Chewsa Kebele 5 km south of INJIBARA town between the towns of INJIBARA and KESSA and only 200 m away from the Addis Ababa- Bahir Dar high way at an elevation (altitude) of 2500m. The diameter of the lake is roughly 1 km and it is a closed lake. Zengena is lake with a maximum depth of 166m and it is the second deepest lake in Ethiopia after Lake Shalla and followed by Tirba (maximum depth of 153m) which is also found in this zone. Its rim is made up of unconsolidated ash deposits. Zengena Lake is most likely a maar lake formed by volcanic explosion and collapse. Though it is not proved, as villagers uttered, the formation of this Lake has a myth that is told by the community, which is similar with the biblical history of Noah and floodwater. This lake is used as a holy water. The water sample was collected from the Lake Zengena located at 10°54'47.7"N,36°57'56.4"E /10.91389°N 36.96667°E in the Banja woreda of Awi zone of the Amhara Regional state in Ethiopia[97]. The Lake Zengena is located 433.6 km far from Addis Ababa, which is the capital city of Ethiopia and 120 km away from Bahir Dar, the capital city of Amhara regional state. Water samples were collected from Zengena Lake for the study. The lake contains poorly developed natural vegetation exists around the crater on the steeply sloping sides. The lake serves as lives or host for animals and birds. Animals like, Abyssinian Colom bus Monkeys, Hyena, Anubis baboon, Common bush buck and others animals found around the lake and some fish types as resilient Tilapia occurs in the lake. Then the physico-chemical characteristics of the sample was collected from the Lake i.e. from site one (Zingini) and from site two (Chewssa) to determine its parameters and some heavy metals. These sites were selected due to their differences in their position of the chance the contaminant can enter. The distance from the two sites is around 500m. Moreover, the distance from replicate sites is 10m far apart each other and there are 6 in number. Some water parameters should be studied in the meantime when taking a sample like temperature and others should be studied in the laboratory.

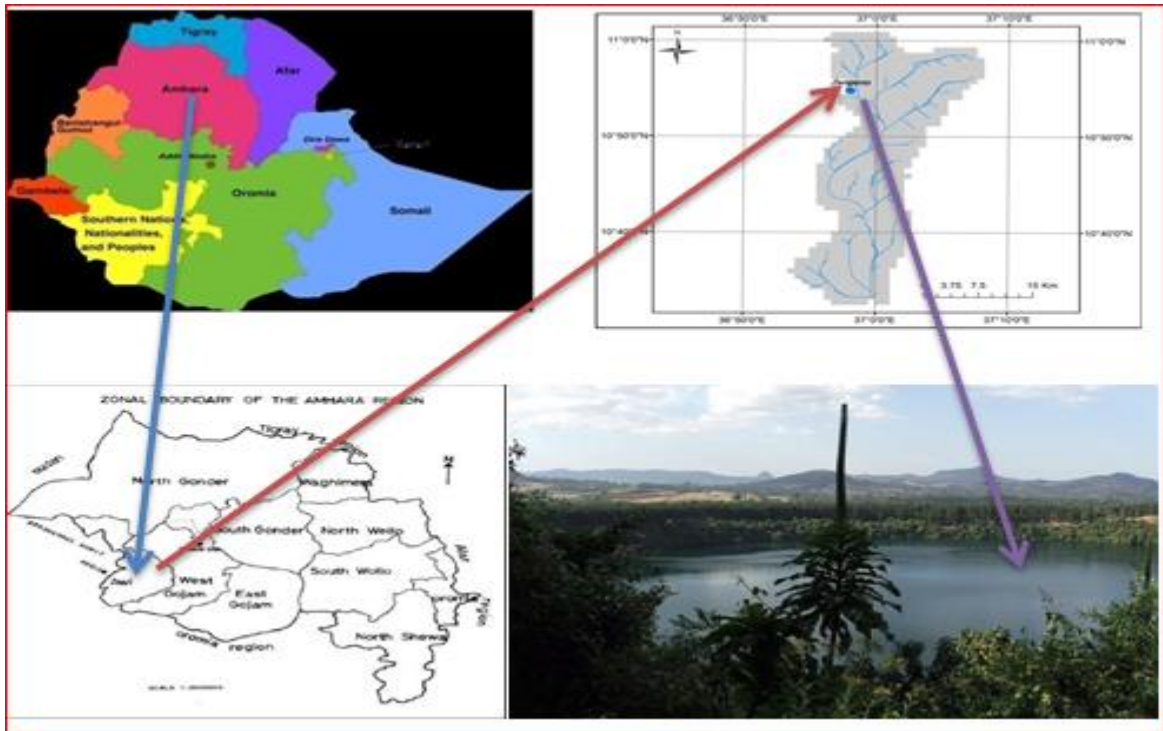


Figure 1:-Location of lake Zengena on the map of Ethiopia,

3.2. Sampling

Purposive sampling was used in this study where the cases best contributing to the information needs of the study were selected. Water samples were collected from lake Zengena, Banja Woreda, Awi Zone Ethiopia. Sample collection, transportation, and storage should be conducted in accordance with international standards (ISO) [98]. Before sample collection, sampling polythene plastics were thoroughly washed, rinsed and dried and were labeled with site of collection and dates. For all the physico-chemical parameters, sample collection was taken in triplicate from selected sites. Water sample from the lake were collected separately from site in two liter polythene plastics and transported to Hawassa University analytical chemistry laboratory for pre-treatment. The determination of heavy metals and physico-chemical parameters was done at Hawassa University analytical chemistry laboratory and Environmental engineering laboratory. Before sample analysis in the laboratory, the collected sample was preserved in the refrigerator at $\leq 6^{\circ}\text{C}$ [99].



Figure 2:-Sample from the lake Zengena, source researcher's own (on, 25/07/2024 G.C)



Figure 3:-Sample site one, source researcher's own (on, 24/07/2024 G.C)



Figure 4:-sample site two, source researcher's own (on, 24/07/2024 G.C)

3.3. Chemicals and Reagents

The reagents used in the study were all analytical grades. They include; Nitric acid (69-72%), from Loba Chemie Pvt. Ltd (Mumbai, Maharashtra, India), H₂O₂ (30%), from RdH Labor Chemikalien GmbH and CO.KG (Seelze, Germany), and HCl (37%) from Loba Chemie Pvt. Ltd (Mumbai, Maharashtra, India), were used for the digestion of the water samples. The stock standard solutions (Buck Scientific puro graphics calibration standards, USA) containing 1000 mg/L in 2% HNO₃ of the metals (Ni, Cu, Zn, Pb, Ca and Cr) and physico-chemical parameters for experiment. Moreover, tablets (Alkaphot, Nitricol, Palin test fluoride tablets,1&2, Palin test hardicol 1&2, Palin test phosphate 1&2 LR tablets and Alkaphot P & M) were used to test nutrient ions were also used. Distilled-deionized water was used throughout the experiment for sample preparation and dilution and rinsing of apparatus prior to analysis.

3.4. Instruments and Apparatus

Atomic Absorption Spectrometer, AAS (Buck, scientific model – VGP - 210) equipped with deuterium background corrector and air-acetylene flame atomizers were used for determination of concentration of selected metals. In addition, were used to determine the concentration of the selected metals (Ni, Cu, Zn, Pb, Ca and Cr) and Palintest automatic wavelength selection photometer or Photometer (Wagtech, Scientific model 7100, HANAN product.UK) was to test some water parameters directly. Thermo Fisher Scientific K.K. AQ4500 Handy type Turbidometer Orion Aqua fast (0-4000NTU, EPA (0-2000NTU)) model was used to measure turbidity. AD8000 professional multipara meter pH/ORP/EC/TDS Temp Bench meter was also to measure EC, and TDS. In addition, Hanna instruments pH 211-microprocessor pH meter was used to measure pH of the sample. Hot plate (986083 USA) for digestion, a refrigerator (Hitach Japan) used to keep the collected samples before and after digestion. Also ,burette, micropipette, stirrer, base stand ,clamp, sample digester, beakers, funnel, 500ml plastic bottles, 100m volumetric flask, round test tube10ml glass was used to measure parameters of water and heavy metals.

3.5. Analysis of Physico-Chemical parameters

3.5.1. Temperature

The temperature of Lake Zengena water samples was recorded on the sampling sites with help of a portable thermometer i.e., Celsius thermometer.

3.5.2. pH

Onsite analysis of the physical parameters such as temperature, electrical conductivity, pH, TDS and turbidity were carried out at the site of sample collection according to the standard protocols and methods of American Public Health Association (APHA).

The pH it was measured using pH meter. Before taking the measurements, the pH meter were calibrated, with standard solutions (pH 4.0 and 7.0) and the value of each sample was taken after immersing the pH probe in the water sample and holding for a couple of minutes to achieve a stabilized reading. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross contamination among different samples.

3.5.3. Electrical Conductivity

The conductivity of water was determined with the aid of Conductivity meter and articulated in terms of $\mu\text{s/cm}$. The probe was calibrated using a standard solution with a known conductivity. The probe was immersed in the water sample and the reading was recorded after the disappearance of stability indicator. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross contamination among different samples.

3.5.4. Turbidity

The turbidity of water sample was directly determined by the portable turbidity meter (Jenway (6035) at room temperature. Each sample was poured in the sample holder and kept inside for a few minutes. After achieving the reading stability, the value was recorded. Their measurements were taken immediately after the samples were collected on each site.

3.5.5. Total Dissolved Solids (TDS)

Total Dissolved Solids (TDS): A filtered water sample quantity of 50ml was oven-dried in a beaker. The difference in the weight of the empty beaker and the oven dried content can be estimated as the TDS[100]. However, in this study, TDS was measured by TDS meter directly 3 times and its mean was taken or recorded.

3.5.6. Alkalinity

Total alkalinity is an important test in determining the aggressiveness or scale-forming tendency of the water. If the total alkalinity is low, the water may be aggressive and cause corrosion to pipe work and structures; if the total alkalinity is high, the water may more

readily promote scale formation. Alkalinity control is therefore an important part of many water treatment programs. Alkalinity Values can be determined by titration methods[101].

Alkalinity was determined by photometric method. In the photometric method or Palintest method by using Palintest Alkaphot Tablets, 10mL round test tube was filled with sample. Moreover, one Alkaphot tablet was added, crushed, and mixed until all particles to be dissolved and by selecting photometric wavelength of photometer displayed its reading and it was recorded. However, alkalinity M and P calculated from the sample was 90mg/L and 25mg/L respectively. These values of M and P are used to calculate alkalinity by using formula.

3.5.7. Determination of Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand was determined using azide modification of Winkler's method. BOD bottle was prepared and incubated at 20°C for 5 days in the dark. After five days, incubated BOD bottle was poured with mixing 2mL of orthophosphoric acid; it was shaken gently and titrated with sodium thiosulphate to the end where there was change in colour. Titre value represents dissolve oxygen on day five. BOD was then calculated as the difference between dissolve oxygen on day one and that on day five.

3.5.8. Determination of Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) test is used to indirectly measure the amount of organic compounds in water. Most application of COD is to determine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in mg/L, which indicates the mass of oxygen consumed per liter of solution. COD is the measurement of the amount of oxygen in water consumed for chemical of pollutants. COD determines the quantity of oxygen required to oxidize the organic matter in water or wastewater sample, under the specific conditions of oxidizing agent, temperature, and time. This method covers the determination of COD in ground and surface waters, domestic and industrial wastewaters. The applicable range is 3-900mg/L.

Determination of COD was done as per the method described in standard methods. 50 ml of the water sample was taken in a reflux flask, and 10mL of potassium dichromate solution with 1 g mercuric sulphate was thoroughly mixed. Antidumping beads were added to control boiling of the solution. To this, 10mL of concentrated sulphuric acid containing silver sulphate was added through the open end of the condenser carefully and mixed by swirling motion, reflux apparatus was operated for around 1 hour and allowed to cool flask was removed, and its content was diluted to 150mL with distilled water. To the resulting solution,

three drops of the ferroin indicator were added. The sample was titrated with standard ferrous ammonium sulphate to an end where blue-green colour just changed to reddish-brown. Chemical oxygen demand (COD) of the blank sample was then calculated.

3.5.9. Dissolved Oxygen

Dissolved oxygen was determined using azide modification of Winkler's method. 200mL of the water sample was carefully transferred into a 300 ml BOD bottle. 1mL of manganese sulphate solution was added followed by 1mL of the alkaline alkali-iodide-azide reagent. Resulting mixture was titrated against 0.025N sodium thiosulphate to the end where there was colour change. Then the titre value was recorded as DO[102].

3.5.10. Determination of Nitrate

Determination of nitrate was done by the Palintest nitrate test method, in this method nitrate was reduced into nitrite. The resulting nitrite is then determined by a diazonium reaction to form a reddish dye. The reduction stage is carried out using the unique zinc-based Nitrate Powder, and Nitrate Test Tablet, which aids rapid flocculation after the one-minute contact period. The nitrite resulting from the reduction stage was determined by reaction with sulphanilic acid in the presence of N-(1-naphthyl)-ethylene diamine to form a reddish dye. The reagents are provided in a single Nitricol tablet that is simply added to the test solution. The intensity of the colour produced in the test is proportional to the nitrate concentration and is measured using a Palintest Photometer. Nitrate test tube was filled with 10ml of sample, and one level spoonful of nitrate powder and one nitrate test tablet and shake tube well for one minute. Moreover, one Nitricol tablet was added, crushed, and mixed to dissolve. Then, stand for 10 minute to allow full color development. After this, select phot 23 on photometer for result as mg/L N, or phot 63 for result as mg/L NO₃. To convert mg/L N to mg/L NO₃ multiply result by 4.4. Now, by taking photometer reading the result was recorded by following Photometer instructions. Then, the colour intensity (absorbance) was measured at a wavelength of 660nm in a spectrophotometer[103].

3.5.11. Determination of Nitrites

Determination of the nitrites was done by using the Palintest Nitricol tablets and Palintest Automatic Wavelength Selection Photometer. By using round test tubes and 10mL glass 10mL sample and adding one Nitricol tablet, and crushing it to dissolve and allowing it for 10

minutes, the full colour change was developed. In addition, selecting photometer wavelength it reads amount of nitrites.

3.5.12. Determination of Fluorides

Determination of fluoride was done by using Colorimetric method (Using Palintest Fluoride tablets) and Palintest Photometer[104]. By using round test tubes and 10mL glass (PT515), and filling the test tube with 10mL sample and it was marked. Then, one fluoride number one tablet was added, crushed, and mixed to dissolve. Again, the second number one fluoride tablet was added then crushed, and mixed to dissolve. Later, 5 minutes standing to allow full colour development and by selecting the wavelength at 570 nm on the photometer. Then photometer reading was taken.

3.5.13. Determination of Hardness

Determination of hardness was done by using Palintest photometric method by using hardicol test tablets[105]. Hardness of water was measured by using hardicol test tablets. The Palintest Hardicol test is based on a unique colorimetric method. The reagents are provided in tablet form and the test is carried out simply by adding the appropriate tablets to a sample of the water. Under the controlled conditions of the test calcium and magnesium, ions react with hardicol indicator to produce a purple coloration. The intensity of the color is proportional to the total hardness of the water and is measured using a Palintest photometer. Here, round test tube and 10mL glass (PT 515) and Palintest hardicol number 1 and 2 tablets were used. Therefore, the sample was filtered and test tube was filled with sample. Hardicol number 1 tablet was added, crushed and mixed to dissolve. In addition, hardicol number two tablets was added, crushed and mixed to dissolve. Then complete dissolving of all particles was ensured. By standing or keeping 2 minutes up to full color development and selecting the wavelength 570nm on the photometer it displays the reading. Therefore, the photometer displayed the reading of it.

3.5.14. Determination of Phosphates

Determination of Phosphate was done by using Palintest photometer colorimetric instrument by a method of Palintest phosphate LR method. Palintest photometer, round test tube and 10mL glass (PT 515) and Palintest phosphate number 1 and number two LR tablets were used. Therefore, 10ml sample was added to test tube and phosphate number 1 &2 LR tablets were added, crushed, and mixed to dissolve. After 10 minutes to allow full color development wavelength of 640nm was selected/adjusted on the photometer. Therefore, photometer gives its reading.

3.5.15. Determination of Carbonates

Determination of carbonate was also done by using Palintest photometer reading depending on the alkalinity calculated above. The alkalinity was calculated as alkalinity P and alkalinity M.

For Alkalinity P, the test tube was filled with 10mL of sample and one Alkaphot P tablet was added, crushed and mixed to dissolved and stand for two minutes to allow complete color development and then wavelength was adjusted to 520nm on the photometer and photometer was displayed its reading and taking Alkaphot P calibration chart.

For Alkalinity M, the test tube filled with 10ml of sample and one Alkaphot M tablet was added, crushed and mixed by ensuring all particles were dissolved. Then, the wavelength was adjusted to 570nm on the photometer and taking photometer reading and Alkaphot calibration chart photometer displayed the reading.

Having above results and by using the following relationship between P&M,

- ✓ If alkalinity $P=0$, then Bicarbonate= M
Carbonate= 0
Hydroxide= 0
- ✓ If Alkalinity $P>0$ and $M>2P$, then Bicarbonate= $M-2P$
Carbonate= $2P$
Hydroxide= 0
- ✓ If alkalinity $P>0$ and $M<2P$, then Bicarbonate = 0
Carbonate= $2M-2P$
Hydroxide= $2P-M$

3.5.16. Determination of Bicarbonates

Determination of bicarbonates was also done by using Palintest photometer reading depending on the alkalinity calculated above. The alkalinity was calculated as alkalinity P and alkalinity M.

For Alkalinity P test tube was filled with 10mL of sample and one Alkaphot P tablet was added, crushed and mixed to dissolved and stand for two minutes to allow complete color development and then wavelength was adjusted to 520nm on the photometer and photometer was displayed its reading and taking Alkaphot P calibration chart.

For Alkalinity M, the test tube filled with 10ml of sample and one Alkaphot M tablet was added, crushed and mixed by ensuring all particles were dissolved. Then, the wavelength was adjusted to 570nm on the photometer and taking photometer reading and Alkaphot calibration chart photometer displayed the reading.

Having above results and by using the following relation ship

- ✓ If alkalinity $P=0$, then Bicarbonate= M
Carbonate= 0
Hydroxide= 0
- ✓ If Alkalinity $P>0$ and $M>2P$, then Bicarbonate= $M-2P$
Carbonate= $2P$
Hydroxide= 0
- ✓ If alkalinity $P>0$ and $M<2P$, then Bicarbonate = 0
Carbonate= $2M-2P$
Hydroxide= $2P-M$

The expression of alkalinity results sometimes causes confusion. It is normal practice to express the result as mg/L CaCO_3 . This is merely a convention to allow the comparison of different results and does not necessarily indicate that the alkalinity is present in the water in this form.

3.6. Digestion of Water Samples

Digestion of water took place after composite samples were prepared from the Lakes. According to the Methods developed by the United State Environmental Protection Agency (USEPA) a 100 mL aliquot of well mixed water samples were digested in a beaker covered with a watch glass by adding 1 mL of concentrated (69-72%) HNO_3 and 3 mL of concentrated (30%) HCl and heated on a hot plate at 90°C boiled until the solution reached up to the mark (20 mL). Then the beaker was removed and cooled. Each of the digested water samples was filtered through Whatman filter paper No.42 in to a 50 mL volumetric flask and filled up to the mark with deionized water.

3.7. Metal Analysis

Cr, Ni, Cu, Zn, Pb and Ca were determined using Flame Atomic Absorption spectroscopy (Buck Scientific, Model 210VGP AAS, USA) equipped with deuterium background corrector and used air-acetylene flame atomizer. Stock standard solutions (Buck Scientific purographics calibration standards, USA) containing 1000 mg/L of the metals Cr, Ni, Cu, Zn, Pb, Mg, K, Fe and Ca from which 10 mg/L of intermediate standard obtained were used for preparation of calibration standards of each metal.

3.8. Instrument Operating Conditions and Calibration

Metals were analyzed using flame atomic absorption spectrophotometer with external calibration curve after the parameters such as burner and lamp alignment, slit width and wavelength adjustment were optimized for maximum signal intensity of the instrument.

Table 2:-Instrumental Operating Conditions for Determination of Selected Metals in water sample using FAAS.

Note: nm=nanometer, mA=mill ampere, eV=electron volt

Metals	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Energy (eV)	Instrumental detection limit (mg/L)	Flame, oxidant & fuel type
Cr	357.9	0.7	2.0	2.712	0.040	Air-acetylene
Ni	341.5	0.2	7.0	2.624	0.020	Air-acetylene
Cu	324.7	0.7	1.5	3.938	0.005	Air-acetylene
Zn	213.9	0.7	2.0	3.237	0.005	Air-acetylene
Pb	283.2	0.7	2.0	2.874	0.040	Air-acetylene
Ca	422.7	0.7	2.0	3.912	0.010	Air-acetylene

3.9. Analytical Method Validation

3.9.1. Calibration Curve for metals analyzed by using FAAS

The correlation coefficient for the calibration curves for some selected metals analyzed by using FAAS were greater than or equal to 0.999 which assured the linearity of instrumental response for individual metals or these correlation coefficients showed that there was a very good correlation (linear relationship) between concentration and absorbance. The equation of the graph and its R^2 value for some metal are shown below table. The calibration graphs for selected metals analyzed were shown in the appendix.

Table 3:-Concentration of the standard solutions used to establish calibration graphs for the determination of metals from the water samples and their corresponding correlation coefficients.

Metal(analyte)	Intermediate standard concentration by ppm	Standard concentration by ppm	Calibration equation	R ² value
Zn	10	0.5,1,1.5,2,2.5,3,3.5,4,4.5	Y=0.0099x+0.0001	0.9997
Pb	10	0.2,0.4,0.6,0.8,1,1.2,1.4	Y=0.029x+0.0006	0.0006
Ni	10	0.2,0.4,0.6,0.8,1,1.2	Y=0.2266x+0.0016	0.9994
Cr	10	0.5,1,1.5,2,2.5,3,3.5	Y=0.0239x+0.0023	0.9990
Cu	10	0.2,0.4,0.6,0.8,1,1.2	Y=0.0892x	0.9994
Ca	10	0.01,0.02,0.03,0.04,0.05,0.06	Y=0.8877x	0.9992

3.9.2. Method detection limit

Method of detection limit is the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from the blank results. The method detection limit values for the selected metals analysed were above the instrumental detection limit values listed in the manufacturer's installation, operation and maintenance manual. This showed that the concentration of the selected metals in the samples analysed was quantified with an acceptable degree of confidence using the instrument-flame atomic absorption spectrometer. For this research, six blank samples were digested following the same procedures utilized for digesting the water samples. Each blank was assayed for its metal contents (Cr, Cu, Zn, Ca, Ni and Pb) by FAAS.

$$MDL= 3SD\text{-----} (1)$$

3.9.3. Recovery Test

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. In present study due to the absence of certified reference material water in our laboratory, the validity of the digestion procedure, precision and accuracy of FAAS was assured by spiking

water samples with standard of known concentration. The spiked and non-spiked water samples were digested following the same procedure employed in the digestion of the respective samples and analyzed in similar condition. Then the percentage recoveries of the analytes were calculated the following formula.

$$Recovery = \frac{C(spiked) - C(non\ spiked)}{C(added)} \times 100\% \text{-----} (2)$$

3.9.4. Discussion of physico-chemical parameters

Physico-chemical parameters are discussed also by comparing with compulsory Ethiopian Standards (CES) in addition to WHO.

3.9.5. Method of Detection Limit

Method of detection limit is the minimum measured concentration of analyte that can be measured. In other words, it is the lowest analyte concentration that can be distinguished from statistical variations in a blank, which usually correspond to the signal of blank three times the standard deviation of the blank (3δ blank, where δ = standard deviation of the blanks). As shown in Table 5, the method detection limits of the investigated elements by FAAS along with their instrument detection limits are presented, respectively.

Table 4:- Instrument detection limit & method detection limit

Element	IDL (mg/L)	MDL(mg/L)
Calcium	0.010	0.5
Zinc	0.005	0.01379
Copper	0.005	0.01196
Nickel	0.020	0.06
Chromium	0.040	0.07309
Lead	0.040	0.04489

Where, MDL method of detection limit and IDL is instrument detection limit

3.9.6. Statistical Analysis

In this study, Microsoft Excel was used for statistical analysis. Statistical evaluations such as mean, standard deviation and relative standard deviation have done for all parameters of water. All analysis was carried out in triplicate. Methodological precision was there evaluated with standard deviation (SD). Statistical analysis of the data were carried out using t-test was computed to assess significant variation in the mean concentration of selected metals in the water samples. In addition, Origin was used to analyze the calibration curve for metals selected from the sample. The least significant difference test of probability level of $P < 0.05$ or 95% confidence level was considered statistically significant.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

In this study, the results of analysis of physico-chemical characteristics and selected heavy metals of water samples and all measured parameters was summarized in the tables below and values of each were discussed. In addition to these, comparison of physico-chemical parameters and heavy metals of studied water samples had also been tried to compare with WHO guide line values of drinking water and other standards.

4.1. Instrument Calibration

Calibration curve for metals analysed using AAS: - The correlation coefficients for the calibration curves of all selected metals analysed using FAAS were equal to or greater than 0.999. This indicates a strong linear relationship between concentration and absorbance for each metal. Below are the equations of the graphs along with their respective R^2 values for each metal were also shown in the table 3. The calibration curves for each analyte of interest are also given in appendix 2.

4.2. Physico-chemical characteristics of Lake Zengena

Table 5:-Physico-chemical characteristics of Lake Zengena and WHO maximum permissible level in mg/L in a drinking water

No	Parameters	site one result (n=3mean±standard deviation)	Site two result (n=3mean±standard deviation)	WHO	Reference
1	Temperature ($^{\circ}$ c)	20.63 ± 0.057	20.13 ± 0.0416	NL	
2	pH	6.82 ± 0.01	6.62 ± 0.00816	6.5-9.5	[31]
3	Electrical conductivity (μ s/cm)	203.3 ± 0.15	199.1 ± 0.471	<1000	[31]
4	Total dissolved solid (ppm)	20.5 ± 0.1	18.23 ± 0.124	500	[31]
5	Alkalinity (mg/L)	90.16 ± 0.11	80.26 ± 0.124	20-200	[31]
6	Turbidity (NTU)	0.52 ± 0.015	0.41± 0.00816	1.5	[31]
7	Dissolved oxygen (mg/L)	7.16 ± 0.11	6.3 ± 0.0816	NL	

8	Biological oxygen demand (mg/L)	2.46 ± 0.05	2.3 ± 0.0816	6	[31]
9	Chemical oxygen demand (mg/L)	8.56 ± 0.057	9.16 ± 0.0471	10	[31]
10	Fluoride (mg/L)	0.98 ± 0.0057	0.726 ± 0.00471	1.5	[31]
11	Nitrate (mg/L)	2.73 ± 0.0057	2.72 ± 0.0816	<50	[31]
12	Nitrite (mg/L)	0.036 ± 0.0057	0.034 ± 0.0081	3	[31]
13	Total hardness(mg/L) CaCO ₃	44.5 ± 0.1	41.4 ± 0.081	10-500	[31]
14	Phosphates(mg/L)	0.99 ± 0.002	0.98 ± 0.074	NL	
15	Carbonates(mg/L) CaCO ₃	50.23 ± 555	51.00 ± 743	NL	
16	Bicarbonates(mg/L) CaCO ₃	51.98 ± 257	52.84 ± 115	NL	

Where, NL means not limited by WHO

4.3. Physico-Chemical parameters

4.3.1. Temperature

Temperature (°C) is important for its effect on other properties of water. Temperature values of water sample collected from Lake Zengena is presented in Table 4, which is in the range of 20.13°C for site one and 20.63 °C for site two.

4.3.2. pH

Hydrogen ion concentration (pH) is the negative logarithmic value of hydrogen ions in the water, which is an indicator of acidity or alkalinity. It is described that when the pH value of the water is very low, heavy metal ions are released into the water bodies, such as cadmium ions (Cd), lead ions (Pb) and mercury ions (Hg). Are elements that are toxic to aquatic organisms, especially fish[106].

In the present study, pH was recorded with mean \pm SD value of 6.82 ± 0.01 for site one and 6.62 ± 0.00816 for site two (Table 4). This result indicates that the water is somewhat neutral compared with the guideline and the result is in acceptable range.

4.3.3. Electrical Conductivity

Electrical conductivity is the ability of the water to conduct electricity and an indicator for the total ionic content of the water, and thus indicates the water is polluted or not polluted[107]. The mean value of the electrical conductivity of the present study was 203.3 ± 0.15 for site one and 199.1 ± 0.471 for site two (Table 4). Similarly, the present study is slightly in agreement with research done in Nigeria by[108]; thus, the results of the present study imply the water of Lake Zengena is not polluted. This indicates the result is below WHO. Therefore, there is no hard for drinking.

4.3.4. Total Dissolved Solids (TDS)

Total dissolved solids (TDS) are solid materials that can be dissolved in water consisting of inorganic and organic compounds in the form of mineral water and their salts. These materials can increase the value of turbidity, which can inhibit light penetration into the waters so that it affects the photosynthesis process[108]. In the present study, the mean value of total dissolved solids $20.5 \pm 0.1\text{mg/L}$ for site one and $18.23 \pm 0.124 \text{ mg/L}$ for site two (Table 4). The result was lower than recommended range by FEPA (500 mg/l), cited at [109]. The present findings are slightly lower than study done by[110]. Total dissolved solids are the indicator of dissolved substances in the water body due to watershed, rainfall and surface runoffs water[109].

4.3.5. Turbidity

Turbidity is the ability of the water to have suspended particles and planktonic organisms, which blocks the amount of light penetration in the aquatic ecosystem. It is the result of planktonic organisms or from suspended particles in the water body. Turbidity may reduce light penetration, thus reducing photosynthesis in the water body[111]. The mean turbidity value of this study was $0.52 \pm 0.015 \text{ NTU}$ for site one and $0.41 \pm 0.00816 \text{ NTU}$ for site two (Table 4).

The result of this study indicates that the lake water was not turbid. This may be due to the presence of fewer amounts of planktonic organisms and fewer suspended materials in the lake.

4.3.6. Total Hardness

Water hardness is a measure of the alkaline earth metals such as calcium and magnesium concentration in water samples. The average mean value of the total hardness of Lake Zengena was 44.5 ± 0.1 mg/L CaCO_3 for site one and 41.4 ± 0.081 mg/L CaCO_3 for site two (Table 4). This indicates the lake Zengena is soft water depending on water hardness description. The ideal total hardness for the growth of fish is between 30-180 mg/L, above and below this is stressful to the fish [111]. The result of this study was supported by study [111] that reported total hardness ranged between 80mg/L to 110mg/L..

4.3.7. Alkalinity

Alkalinity was determined by photometric method. From the results obtained from the foregoing procedures, the alkalinity of the sample makes as to give decision about alkalinity of sample. In the present study, the amount of alkalinity of the sample was 90.16 ± 0.11 mg/L CaCO_3 for sample taken from site one and 80.26 ± 0.124 mg/L CaCO_3 for sample taken from the site two (table 4). However, this value is below WHO value and it is safe for use of this water.

4.3.8. Dissolved Oxygen

The amount of dissolved oxygen in waters depends on the organisms surviving in it, because metabolic processes taking place utilizes the dissolved oxygen available[112]. Microorganism in the aquatic environment reduces the amount of dissolved oxygen and it affects the metabolic processes performed by other macro organisms. Other processes like decomposition of dead matter performed by different scavengers in the aquatic environment utilize the dissolved oxygen. Fish needs a minimum of 3-5 mg/L of dissolved oxygen to survive[113]. Conditions of low dissolved oxygen (hypoxia) that occur continuously and acutely have the potential to cause death, and low oxygen concentrations can inhibit growth, food consumption, and the physiological state of fish[114]. In the present study the amount of dissolved oxygen was 7.16 ± 0.11 mg/L for sample taken from site one and 6.3 ± 0.0816 for sample taken from site two (Table 4). The overall mean concentration of dissolved oxygen value was within the permissible limits set by FAO a standard that is greater than 4 mg/L.

4.3.9. Chemical Oxygen Demand (COD)

COD determines the quantity of oxygen required to oxidize the organic matter in water or wastewater sample, under the specific conditions of oxidizing agent, temperature, and time[115]. The COD observed in this study was 8.56 ± 0.057 mg/L for site one and 9.16 ± 0.0471 mg/L for site two (table 4).

4.3.10. Biological Oxygen Demand (BOD)

The BOD is defined as the amount of oxygen used by microorganism in the aerobic oxidation of organic matter. BOD is dissolved oxygen important for microorganism for aerobic decomposition of organic matter present in the water [115]. By considering, BOD as an important parameter in aquatic ecosystem to establish the status of pollution. The BOD observed in this study was 2.46 ± 0.05 mg/l for site one & 2.3 ± 0.0816 for site two (Table 4).

4.3.11. Fluorides

Fluoride occurs naturally in some ground waters. It is toxic to humans and other animals in large quantities, while small concentrations can be beneficial. Fluoride is often added to drinking water supplies if sufficient quantities for good dental formation are not naturally present. Excessive intakes of fluoride can result in discoloration of teeth, which is called mottling. Mottling is relatively common when fluoride concentrations in drinking water exceed 2 mg/L, but it is rare when concentrations are less than 1.5 mg/L[116].

Excessive concentrations of greater than 5 mg/L in drinking water can also result in bone fluorosis and other skeletal abnormalities[117]. Considering, fluoride as important water quality parameters of water. The fluoride concentration observed in this study was 0.98 ± 0.0057 mg/L for site one and 0.726 ± 0.00471 mg/L for site two and from (table 4).

4.3.12. Nitrates

Nitrates are normally present in natural, drinking and wastewaters. Nitrates enter water supplies from the breakdown of natural vegetation, the use of chemical fertilizers in modern agriculture and from the oxidation of nitrogen compounds in sewage effluents and industrial wastes. Nitrate is important to control test for water supplies. Drinking waters containing excessive amounts of nitrates can cause methaemoglobinaemia in bottle-fed infants (blue babies)[118]. The nitrate content observed in this study was 2.73 ± 0.0057 mg/L for site one and 2.72 ± 0.0816 mg/L for site two (from table 4).

4.3.13. Phosphates

Phosphates are extensively used in detergent formulations and washing powders. Phosphates also find widespread application in the food processing industry and in industrial water treatment processes. Agricultural fertilizers normally contain phosphate minerals and phosphates arise from the breakdown of plant materials and in animal wastes. Phosphates can therefore enter watercourses through a variety of routes-particularly domestic and industrial effluents and run-off from agricultural land. Phosphate is an important control test for natural

and drinking waters[119]. The phosphate content obtained from current research was 0.99 ± 0.002 mg/L for site one and 0.98 ± 0.074 mg/L for site two and from (table 4) above.

4.3.14. Nitrites

The nitrite test is also applied for pollution control in wastewaters, and for the monitoring of drinking water[120].

The Palintest Nitricol test provides a simple method of measuring Nitrite Nitrogen levels over the range 0 - 0.5 mg/L N. Higher levels can be determined by diluting the sample. From the current research, the amount of nitrite obtained was 0.036 ± 0.0057 mg/L from side one and 0.034 ± 0.0081 mg/L from site tow and (table 4) above.

4.3.15. Carbonates

Carbonate (CO_3^{2-}) primarily is related with alkalinity of water. Therefore, to measure carbonates it is necessary to calculate alkalinity M and alkalinity P from alkalinity of water. Therefore, current research carbonate content was 50.23 ± 555 mg/L CaCO_3 for site one and 51.00 ± 743 mg/L CaCO_3 for site two and from (table 4) above.

4.3.16. Bicarbonates

Bicarbonates like that of carbonates are related with alkalinity of water. Therefore, to measure bicarbonates it is necessary to calculate alkalinity M and alkalinity P from alkalinity of water. Therefore, the current research bicarbonates content was 51.98 ± 257 mg/L CaCO_3 for site one and 52.84 ± 115 mg/L CaCO_3 for site two from (table 4) above.

All the physico-chemical parameters results are under the conditions that they must be found.

4.4. Recovery of the Experimental Procedure

The procedures used in the current study were validated by spiking experiments for recovery determination, which is usually helpful in the absence of certified reference material to evaluate the accuracy of the method applied for the analyte determination. This was performed by carefully spiking the standard solution prepared, in to water samples to evaluate the extent to which the standard added could be recovered after determination through sample preparation. The percent recoveries was calculated after small and known amounts of the heavy metals from the stock solutions were added or spiked in to the water samples to be digested and after concentration of both spiked and non-spiked samples were read. Results were obtained through calculation. Method validation is the process of providing that analytical method is acceptable for its intended purpose. As shown in (Table 6) below, the

percentage recovery for the water samples lie in the range of 88.33 – 101 %, which are within the acceptable range(80%-120%) for each metal suggesting that the digestion procedure used and the method of analysis were accurate and valid.

Table 6:-Recovery test for the analyzed metals

Metals	Concentrations in the sample(ppm)	Amount added (ppm)	Concentrations in spiked sample (ppm)	% Recovery	RSD
Mg	0.31±0.020	0.2	0.5± 0.01	95± 4.08	4.297
Calcium	0.63±0.02	0.2	0.806±0.015	88.33 ± 6.23	7.059
Zinc	1.65± 0.01	0.2	1.84±0.01	96.66 ±2.35	2.438
Potassium	5.87±0.1	0.2	6.06±0.025	98.33± 0.27	10.448
Iron	0.13±0.02	0.2	0.32±0.01	93.33±2.35	2.525
Copper	0.67±0.015	0.2	0.87±0.01	98.33±2.357	2.396
Nickel	0.003±0.0001	0.1	0.104±0.001	101±0.816	0.808
Chromium	0.03±0.0036	0.1	0.133±0.015	100 ± 8.164	8.164
Lead	0.024±0.001	0.1	0.124±0.001	100± 0.816	0.816

4.5. Concentrations of Metal in Lake Zengena

Table 7:- Concentration of metals in Lake Zengena by ppm and maximum permissible level in the drinking water by ppm and WHO standard

No	Metals	Site one Result (ppm) (n=3) mean \pm standard deviation	Site two Result (ppm) (n=3) mean \pm standard deviation	WHO	Reference
1	Magnesium(mg/L)	0.31 \pm 0.020	0.43 \pm 0.0081	NL	
2	Calcium(mg/L)	0.63 \pm 0.02	0.83 \pm 0.016	NL	
3	Zinc(mg/L)	1.65 \pm 0.01	1.85 \pm 0.0081	3.0	[31]
4	Potassium(mg/L)	5.8 \pm 0.1	4.66 \pm 0.047	NL	
5	Iron(mg/L)	0.13 \pm 0.02	0.12 \pm 0.0081	0.3	[31]
6	Copper(mg/L)	0.67 \pm 0.015	0.76 \pm 0.0244	2	[31]
7	Nickel(mg/L)	ND	ND	0.02	[31]
8	Chromium(mg/L)	ND	ND	0.05	[31]
9	Lead(mg/L)	ND	ND	0.01	[31]

Where, ND means not detected

NL means not limited

This is the maximum permissible level in mg/L of toxic or disease causing substance level in drinking water.

4.5.1. Discussion of metals

From the result presented in the table 7, Zinc, Copper, Potassium Calcium, Iron and Magnesium were detected in water samples whereas Lead, Chromium and Nickel were not detected in the sample.

4.5.1.1. Copper

The concentration of Copper in the water sample was 0.67 \pm 0.015 ppm for site one and (0.76 \pm 0.0244) for site two (Table 7) and (Figure 5) which was within the limit of international

standards (2ppm). The value obtained was higher than other studies conducted in other areas[119, 120]. It is essential element, for enzymes and formation of hemoglobin. However, at high concentration of copper may lead to neurological complications, hypertension, liver, and kidney dysfunctions[121]. No guideline is set by WHO (2017) for Copper content in drinking water.

4.5.1.2. Calcium

Calcium ions are the major constituents of various types of rock and they are the most common constituents present in natural waters ranging from zero to several hundred mg/L[122]. In the present study the concentration of calcium were (0.63 ± 0.02) ppm for site one and (0.83 ± 0.016) for site two (Table 7) and (Figure 5). Calcium ion (Ca^{2+}) can occur naturally in groundwater through the dissolution of carbonate minerals and the decomposition of the sulfate, phosphate, and silicate minerals. High calcium concentrations in water may lead to the formation of solid scales in pipes and kitchen utensils and increased soap consumption.

4.5.1.3. Magnesium

Concentration of magnesium in the water sample was (0.31 ± 0.020) for site one and (0.43 ± 0.0081) for site two (Table 7) and (Figure 5). From the analyzed result, water samples recorded magnesium ions within the permissible limit of WHO and ESA constants, 50 mg/L, for drinking water[123]. The main sources of magnesium in the underground water sampled may be attributed to geological sources such as dolomite and other magnesium containing compounds in sediments and soils of the water samples[124].

4.5.1.4. Potassium

Natural source of potassium in water was from the weathering of rocks, but the elevated quantities in contaminated water may be attributed to the release of wastewater. Potassium levels recorded in the study areas were (5.8 ± 0.1) for site one and (4.66 ± 0.047) for site two (Table 7) and (Figure 5). Concentration of potassium obtained in samples was far below the maximum permissible limit of WHO and ESA constant, 10 mg/L, for drinking water[7].

4.5.1.5. Iron

The concentration of iron measured in water samples were (0.13 ± 0.02) for site one and (0.12 ± 0.0081) for site two (Table 7) and (Figure 5). From the analyzed result, all the samples had iron concentration below the permissible limit of WHO and ESA for drinking water, 0.3 mg/L[7]. Iron may not pose any health hazards but gives a bitter taste to the water

when present in large concentrations. People consuming water sources with high concentration of iron were suffering from taste, color, corrosion of plumbing systems, and liver diseases. However, those exposed to low concentration would be highly susceptible to anemia[124].

4.5.1.6. Zinc

The concentration of zinc in water sample was (1.65 ± 0.01 ppm) for site one and (1.85 ± 0.0081) for site two (Table 7) and (Figure 5). However, the concentrations of zinc in water samples are lower than the EPA standard limit, which is 3 ppm Zinc is a low concentration, and high concentration can cause health problem. Too little zinc can cause slow wound healing and skin sores, decreased sense of taste and smell, loss of appetite and damage in immune system. While in larger amount, for a short period it can causes stomach cramps, nausea and vomiting. For a long period, it can cause anemia, pancreas damage and low levels of high-density lipoprotein cholesterol. Harmful effects generally begin at levels 10-15 times higher than the amount needed for good health[125]. EPA recommended maximum contamination level is 5 mg/L[126].

4.6. Statistical Analysis

Statistical data analysis were done to assess whether if there is significance difference or not by using t- test and the analysis revealed that there was no significance difference between mean concentration of analyzed metals in site one and site two at ($p < 0.05$).

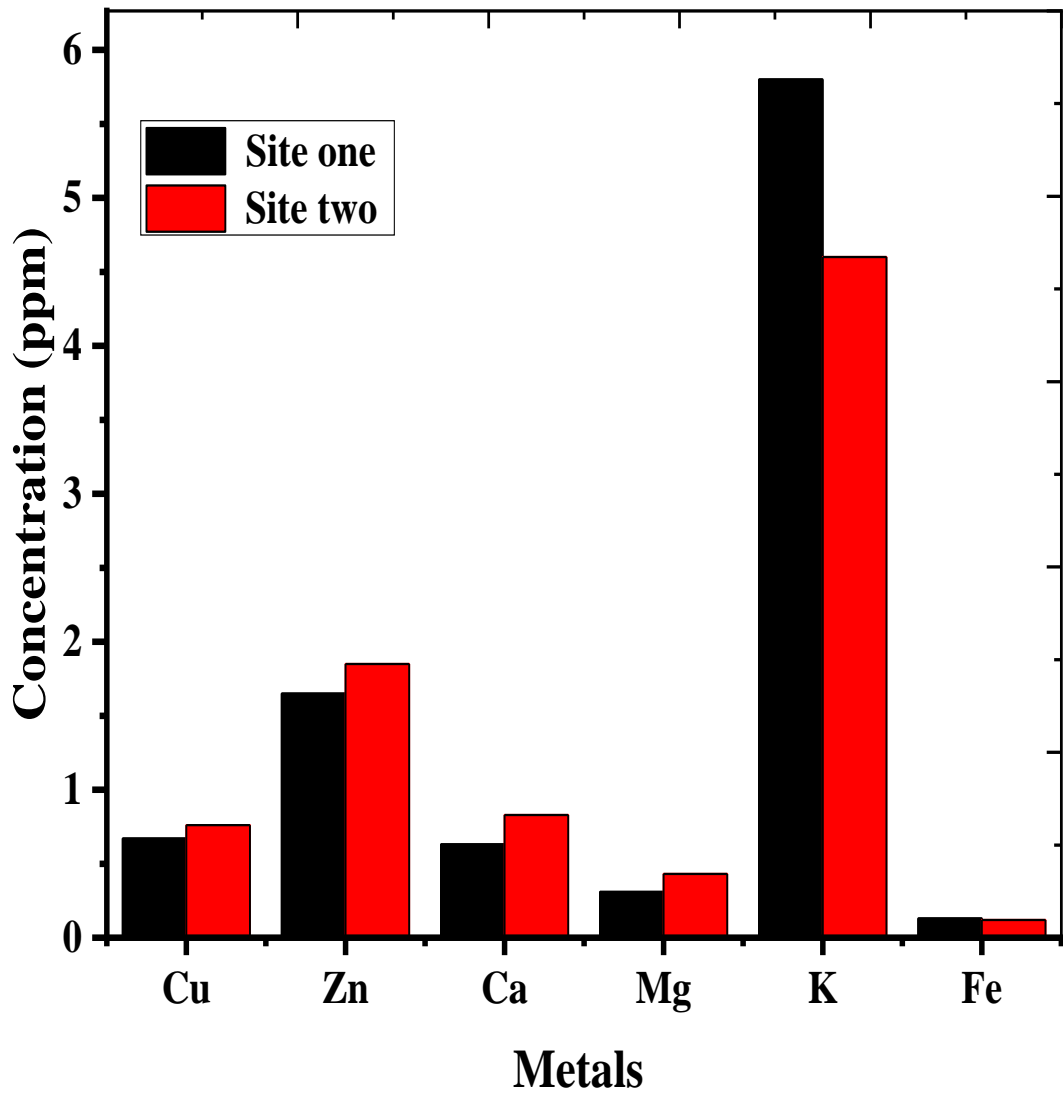


Figure 5:-Distribution pattern of metals (Mg, Ca, Zn, K, Fe, and Cu)

CHAPTER FIVE

5. Conclusion and Recommendations

5.1. Conclusions

The main goal of this research was to assess the status of Lake Zengena water quality of Awi zone in Amhara region, Ethiopia, with the emphasis on physico-chemical parameters and heavy metal analysis. The water sample collected from Lake Zengena was tested for metal (Pb, Cr, Ni, Zn, Cu, Ca, Mg, K, and Fe) and physico-chemical parameters such as temperature, pH, EC, TDS, total hardness, turbidity, alkalinity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, nitrates, nitrites, phosphates, fluoride, carbonates and bicarbonates. These parameters are critical for assessing the ecological health and water quality of lakes. Regular monitoring can help identify changes over time, inform management practices, and ensure the sustainability of these vital ecosystems. Based on the results of this study, heavy metals like Pb, Ni and Cr were not detected. This indicates that there is no suspected source of heavy metal source that can highly damage the lake, which means they are found in a small amount in the lake. However, other analyzed metals Cu, Zn, Ca, Mg, K and Fe were detected and the results are within permissible limit. This indicates also existence of these metals is due to weathering of rocks from the surface of lake. The results of physico-chemical parameters were demonstrating that, most of the parameters were not found averagely to deviate from the standard or found to be present in high concentrations. In addition, most of the water sample parameters were found to be at least at minimal satisfactory level that of the parameters tested to be at acceptable level. The result suggests that the lake Zengena is safe and does not pose any health risk. The distribution of average metal concentrations in the sample water was found in the order of $K > Zn > Cu > Ca > Mg > Fe$.

5.2. Recommendations

Based on the above results the following information's were forwarded:-

- More sensitive analytical instruments should be used to assess the non-detected metals.
- The concerned body and community should take the controlling mechanisms to protect the lake and lakes ecosystem from danger.
- Awareness should be often given to residents.
- The study was conducted with in one season, on the some physico-chemical parameters and heavy metal content. Therefore, it may show lack of comprehensiveness. Further studies should be conducted in different seasons considering other water quality parameters like biological water quality parameters and other water quality parameters of drinking water and irrigation purposes especially fish farm.

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APPENDIX

APPENDIX- 1

Analysis of t-test

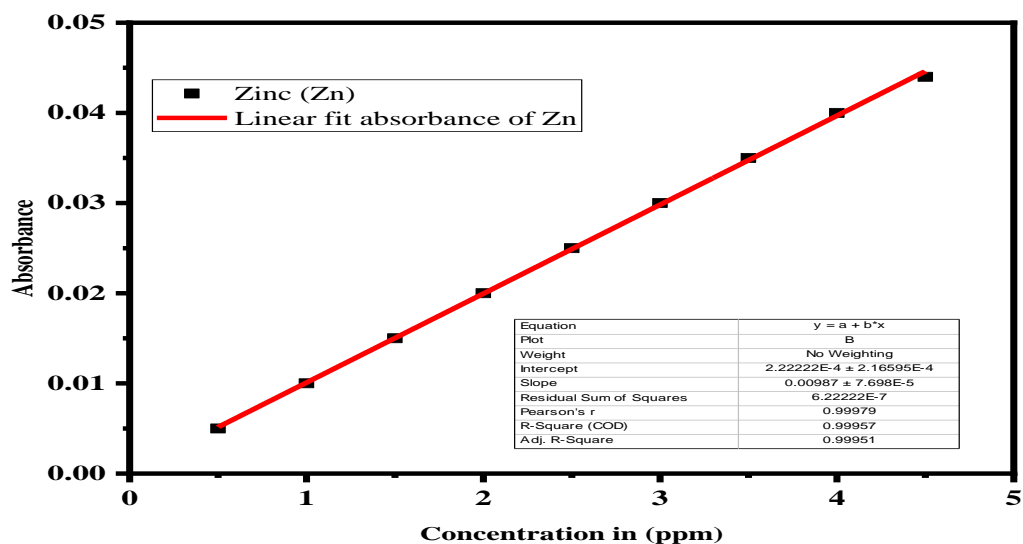
Statistical data analysis were done to assess whether if there is significance difference or not by using t- test and the analysis revealed that there was no significance difference between mean concentration of analyzed metals in site one and site two at ($p < 0.05$).

Table 1:- analysis of metals by t-test

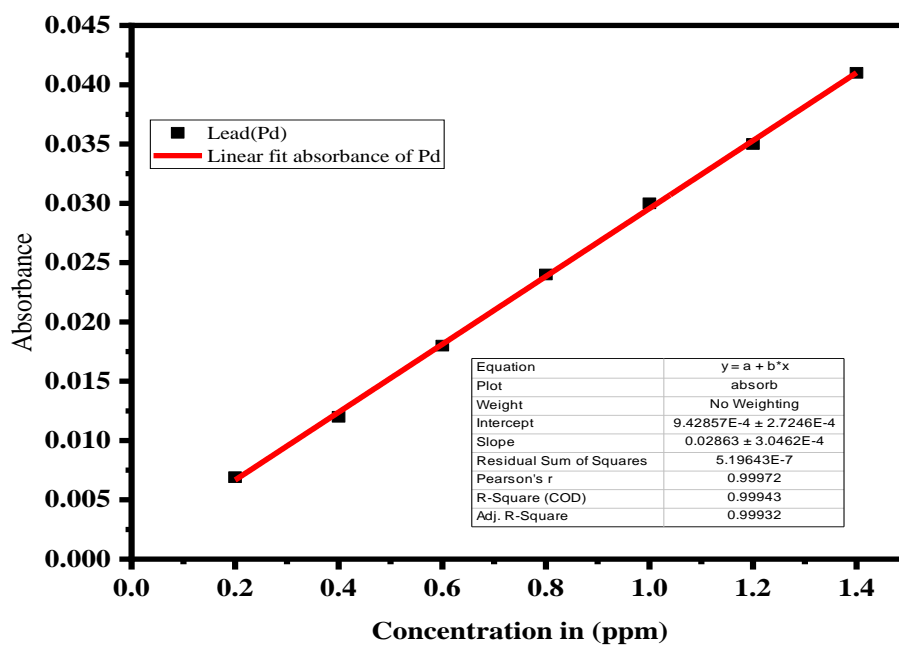
Element(metal)	p-value	df	t-test	t-critical two tail
K	0.026	2	1.5	4.30
Fe	0.042	2	1.0	4.30
Mg	0.019	2	-7	4.30
Zn	0.005	2	-14	4.30
Ca	5.78×10^{-7}	2	-1.3×10^8	4.30
Cu	0.022	2	-6.5	4.30

APPENDIX- 2

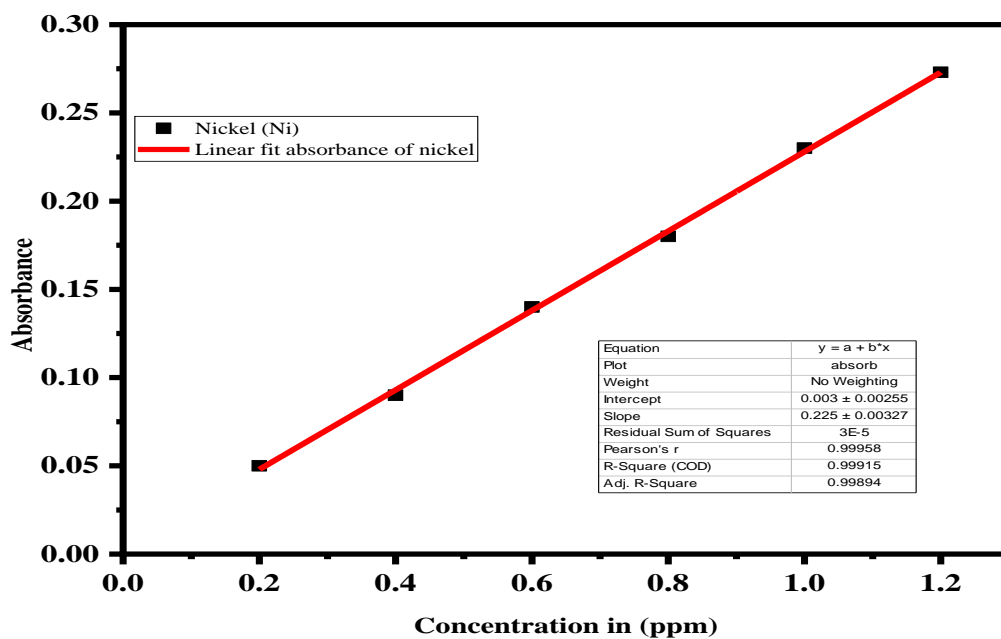
Calibration curve



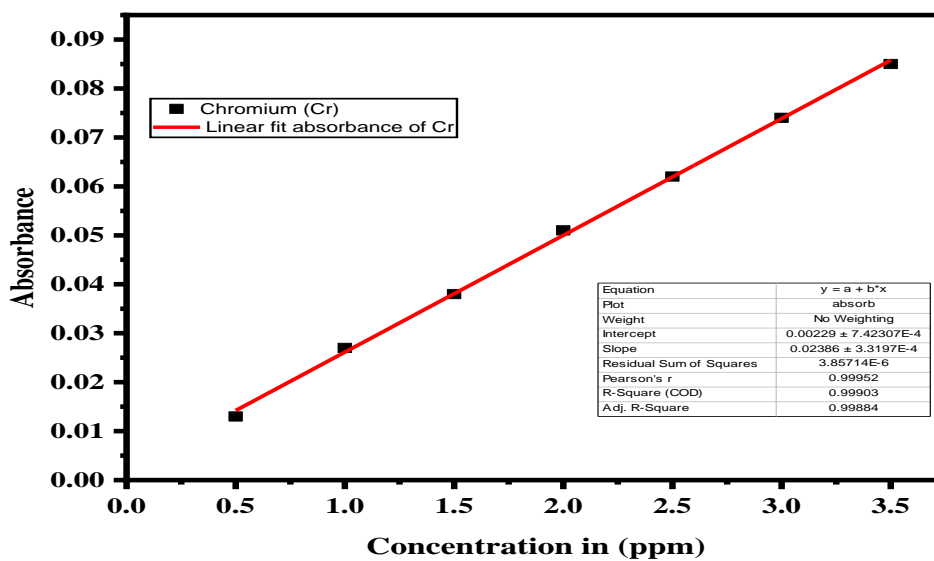
A) Calibration curve for Zn standards



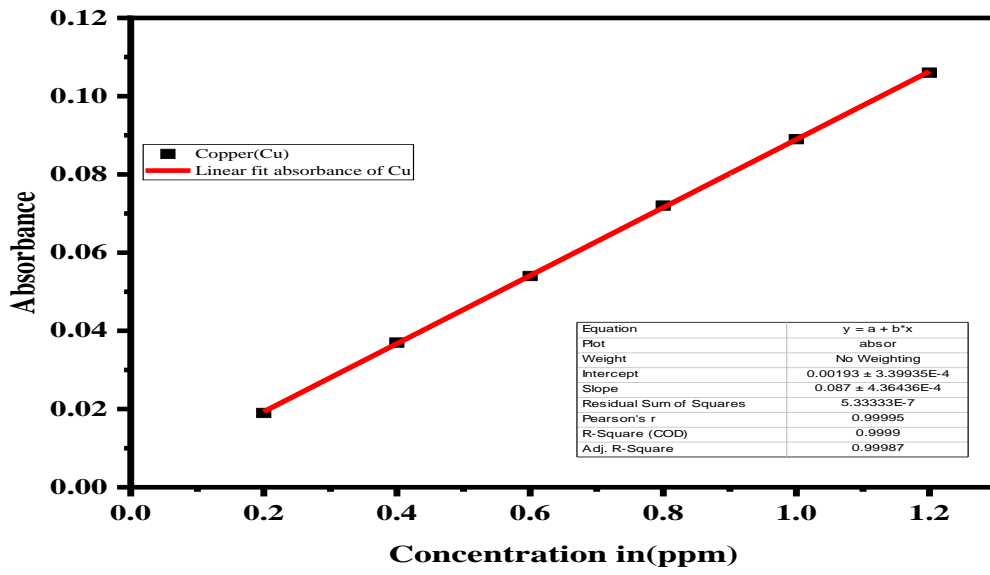
B) Calibration curve of lead standards



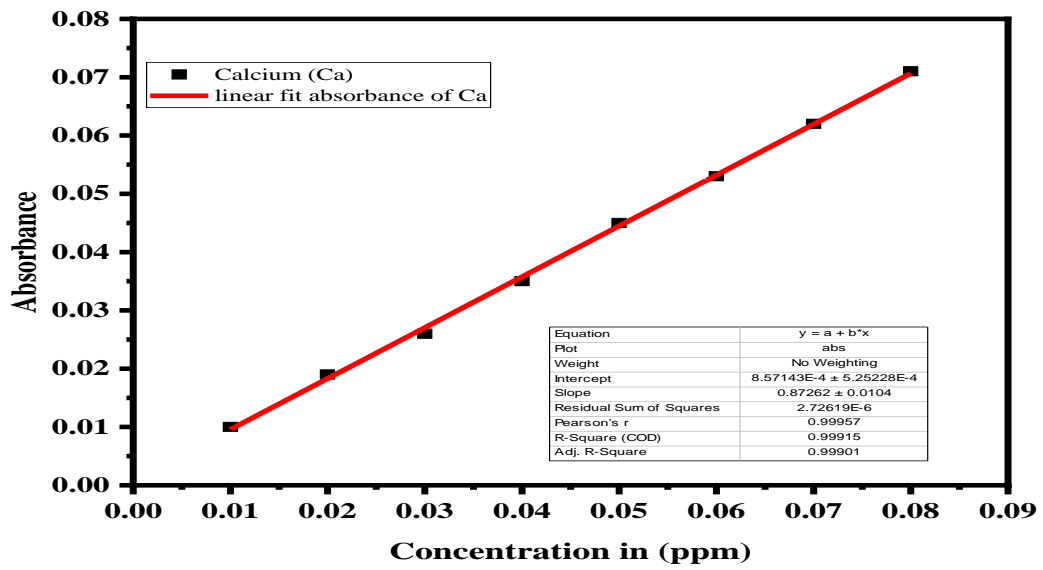
C) Calibration curve of nickel standards



D) Calibration curve of chromium standards



E) Calibration curve of Copper standards



F) Calibration curve of Calcium standards