ATOMIC ABSORPTION SPECTROSCOPIC ANALYSIS OF HEAVY METAL CONTENTS OF MALT BEVERAGES CONSUMED IN ETHIOPIA

MSC IN LASER SPECTROSCOPY PHYSICS

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A THESIS SUBMITTED TO THEDEPARTMENT OF PHYSICS,

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PHYSICS

The undersigned hereby certify that have ready and recommend to the college of Natural and Computational Science, School of Graduate Studies for acceptance a MSc thesis entitled **"Atomic absorption spectroscopic analysis of heavy metal contents of malt beverages consumed in Ethiopia"** submitted authors by Marishet Tessema Tamiru in partial fulfillment of the requirements for the degree of Master's with specialization in Physics (Laser spectroscopy).

Dr. Daniel Mulugeta	_____	_____
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EXAMINERS' APPROVAL SHEET

We, the undersigned members of that the Board of Examiners of the final open defense by Marishet Tessema Tamiru have read and evaluated his thesis entitled "**Atomic absorption spectroscopic analysis of heavy metal contents of malt beverage consumed in Ethiopia**" and examined the candidate. This is, therefore, certifying that the thesis has accepted in partial fulfillment of the requirements for the degree.

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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the School of Graduate Studies (SGS) through the Department/School of Graduate Committee (DGC/SGC) of the candidate's department.

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DECLARATION

I declare that this has not been submitted to any other institution for the award of an academic degree, diploma or certificate. In the preparation, data collection, data analysis, and compilation of this work, I have followed all ethical and technical principles of the thesis. This work was submitted in partial fulfillment of the requirement for the MSc degree physics with a specialization in Laser Spectroscopy at Hawassa University. The experimental work is my own work and the collaborative contributions have been clearly identified and acknowledged.

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ABBREVIATIONS (ACRONYMS)

EMR	Electromagnetic Radiation
AAS	Atomic Absorption Spectroscopy
US-EPA	United States-Environmental Protection Agency
WHO	World Health Organization
FAAS	Flame Atomic Absorption Spectroscopy
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
CVAAS	Cold Vapor Atomic Absorption Spectroscopy
ICP-OES	Induced Coupled Plasma -Optical Emission Spectroscopy
PPM	Part per million
HCL	Hallow cathode lamp
Co	Cobalt
Pb	Lead
Cr	Chromium
Ni	Nickel
Cu	Copper
RSD	Relative standard deviation

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ABSTRACTS

This study was designed to detect the levels of some heavy metals such as, nickel, cobalt, chromium, lead, and copper in five different brands of malt beverages commonly consumed in Ethiopia (Sinq, Sofi, Nigus, Malta Guinness and Bertat), using flame atomic absorption spectrometry (FAAS). The heavy metal contents obtained using FAAS shows slight differences among the five malt beverages. Generally in all brands of malt beverages, the concentration of Cu is the highest and Ni has the lowest concentrations. The order of abundance of these heavy metals is: Cu > Co > Cr > Ni. The levels of Ni and Cr show very small variations among the different brands of malt beverages. On the other hand, concentrations determined for Cu, Co shows slightly higher variations among the different brands. The level of Cobalt (Co) concentrations are in the range between 0.048 mg/L and 0.073 mg/L, which is above the maximum limit that has set by WHO (0.01 mg/L) in five malt beverages. The Ni level obtained in the beverages range between 0.015 mg/L and 0.029 mg/L, which is above the maximum limit set by WHO (0.02 mg/L) except the Bertat that has concentration range 0.015mg/L. The concentration of Cu is in the range from 0.4 mg/L to 0.68 mg/L, while the maximum allowed value set by WHO has 2.0. Therefore the levels of Cu in all the samples are within the acceptable limit. The level of Cr in the samples range between 0.032 and 0.047 which is again below the maximum allowed limit set by WHO (0.05 mg/L). In all samples, the level of Pb is below the detection limit of the instrument. In general, few malt drinks, the level of some heavy metals are slightly above the maximum limit set by the national and international organizations. Therefore, I suggested that malt beverage producing companies should pay attention to continuous monitoring of the sources of these heavy metals in malt beverages. The experimental results are validated in terms of accuracy, precession, linearity and method of detection limit. The accuracy was determined by spiking experiment and an accepted percentage recovery was obtained in the range of 97%-98%.

Keywords: Malt beverage, Heavy metal, AAS, Beer-Lambert's law.

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Drinks are foods that stand apart from other foods primarily because they are liquids that are ingested in their liquid condition and because they can either be consumed for their stimulating or thirst-quenching qualities [1]. They promptly slake thirst and quickly replenish lost energy and salt [2]. None alcoholic beverages are among the frequently consumed drinks. In today's world, the manufacturing and use of non-alcoholic beverages have reached concerning proportions. Among these beverages, malt drinks are becoming the choice of the people around the world. It's a popular beverage in Latin America and the Caribbean, and it's also common in African nations. Since malt drinks are rich in vitamins and other nutrients, it was often given to sick and a young person as sustenance, but it has now become a popular beverage for individuals of all ages [3]. Some health advantages of the beverage include its ability to prevent ulcers, cancer, and coronary heart disease [4]. Malt beverages are often made with barley malt, though they can also be made with wheat, oats, rye, and sorghum in certain situations [5]. In order to make malt drinks, wort granules are typically dissolved in water, filtered, pure hop fragrance is added, and carbonation is then implemented.

In addition to its delightful flavor, malt also offers vitamins, minerals, fiber, phosphates, antioxidants, and a vital means of hydration, but it can also contain high levels of heavy metals. With densities five times higher than water, heavy metals are among the naturally occurring elements with high atomic numbers [6]. Due to their dual nature as essential elements and toxic substances, the heavy metal content in food is of interest. Some heavy metals can be essential at limited amount but some heavy metals are very toxic and harmful even at trace amounts. Small amounts of certain metals, such as iron, chromium, and copper, are necessary for human and animal health. If the body absorbs too much of these metals, issues may arise. Examples of essential elements are iron, zinc, copper, chromium, cobalt, and manganese [7]. Essential heavy metals and their roles have been recently documented [8].The health of humans and other living things benefits from the vital heavy metals. On the other hand, non-essential heavy metals may

be harmful to body cells, even at low doses. Lead, mercury and cadmium are examples of non-essential elements and their toxicity is widely known and acknowledged as a major global health and environmental risk as a result these heavy metals have never desirable in any quantity.

The primary cause of this heavy metal contamination in the food chain is environmental pollution. Two potentially dangerous elements that have caused a great deal of anxiety are lead and cadmium [9]. It has also been noted that weathering and volcanic eruptions are examples of natural events that greatly contribute to heavy metal contamination [10, 11]. Among the industrial sources are refineries that process metal, power plants that burn coal, petroleum refineries, nuclear power plants, high tension lines, plastics, textiles, microelectronics, wood preservation, and paper processing facilities [12]. Heavy metal contamination of food and drink is occurring due to pollution of the land, atmosphere, subsurface and surface waters [13].

The main way that people are exposed to heavy metals is through their diet, so tracking how much of these metals are consumed through food is crucial for measuring their exposure. As soft drink demand such as malt beverages, is so great that production is high and appropriate manufacturing processes can be compromised, which could result in heavy metal pollution. Since these metals are frequently hard for the body to eliminate, they accumulate over time and can lead to illnesses like cancer, heart problems, neurological impairments, lung problems, kidney failure, memory loss, and mental disorders [14]. Therefore, identification and quantifications of these heavy metals in foods and beverages are important for the safety and wellbeing of the consumers. One common technique that is used for heavy metal analysis is spectroscopy.

The most popular method for identifying individual elements in analytical samples is AAS. It uses these elements' ability to absorb light to calculate their concentration using the principle of Beer-Lamberts law. The amount of light absorbed is used to calculate the analyte concentration. In this work AAS with flame atomization process is used to determine the concentration of heavy metals in malt beverage.

1.2. Statement of the problem

Metal corrosion, air deposition, soil erosion, heavy metal leaching, sediment re-suspension, and metal evaporation from water resources in to soil and ground water are additional ways that

environmental contamination can happen[15]. On the other hand, industrial sources include high tension lines, plastics, textiles, microelectronics, wood preservation, paper processing plants contaminated by heavy metals, metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power plants, and high tension lines. Heavy metals are widely diffused throughout the environment due to their numerous industrial, household, agricultural, medical, and technological uses. This raises worries consequence of human health and environmental [16]. Ethiopia has witnessed a growing trend in the consumption of malt beverages, which are known for their refreshing taste and nutritional value. However, concerns arise regarding the potential presence of heavy metals in these beverages, posing potential health risks to consumers. Heavy metals, such as Pb, Ni, and Cu, are known to accumulate in the human body over time and can lead to various adverse health effects, ranging from neurological disorders to organ damage. Despite the increasing popularity of malt beverages in Ethiopia, there is a notable of comprehensive studies assessing the heavy metal content in these beverages. A critical gap in understanding the potential health implications for the Ethiopian population is created by the absence of empirical data on the levels of heavy metals in malt beverages consumed locally leaves. This research aims to address this gap by employing atomic absorption spectroscopy to analyze and quantify the concentration of heavy metals in malt beverages available in the Ethiopian market. By doing so, the study seeks to provide valuable insights into the safety and quality of these beverages, contributing to consumer awareness, regulatory standards, and public health interventions.

1.3. Research questions

The researcher will aim's to address the following key questions:

1. What are the levels of heavy metals, such as lead, cobalt, chromium, copper, and nickel presence in malt beverages available in the Ethiopia market?

2. Are there significant variations in heavy metal levels among different brands or batches of malt beverages?

3. Are the levels of heavy metals in these malt beverages within the acceptable limits set by national or international standards?

1.4. Objectives of the study

1.4.1. General objective

The general objective of this thesis is to analyze the heavy metal content of malt beverages consumed in Ethiopia by using atomic absorption spectroscopic.

1.4.2. Specific objectives

The specific objectives of the thesis work are:-

- To analyze the level of concentration of heavy metal, Lead (Pb), Cobalt (Co), Nickel (Ni), Copper (Cu), and Chromium (Cr) in five different malt beverages.
- To compare the level of five heavy metals among the different brands of malt drinks.
- To compare their concentration in a sample with maximum admissible limit seat by WHO.

1.5. Scope of the study

This study will limit in consider the qualitative and quantitative aspects of five heavy elements, Lead (Pb), Chromium (Cr), Cobalt (Co), Copper (Cu), and (Nickel) in the five different malt beverages.

1.6. Limitation of the study

Due to financial limit and time constraints:

- Only five heavy metals lead (Pb), Cobalt (Co), Copper (Cu), Nickel (Ni), Chromium (Cr), and will be considered in this study.
- The attention will be only the five malt beverages available in local Hawassa markets.
- Limitation of the instrument with respect to time consuming as it analyzes a single element at a time.
- Limitation of the instrument to measure one of the common contaminant toxic heavy metal, mercury (Hg). As mercury needs cold vapor atomization process, the instrument used in this study is not equipped with cold vapor atomizer. That means free Hg atoms

can exist at room temperature and therefore Hg can be measured by atomic absorption without a heated (flame) sample cell.

1.7. Significance of the study

Even at very low concentrations, the heavy metals found in malt beverages are extremely harmful to humans. In many nations, the possibility of metal accumulation from plants growing on contaminated soil is increasingly becoming a bigger concern. Thus, one of the main issues facing environmental and health professionals is the accumulation of heavy metals in soils, water, and plants. This has led to a need for extensive study to be done in order to predict the presence of heavy metals in malt beverages. Paint should pay special attention to harmful metals as Pb, Ni, Co, Cr, and Cu. It is imperative to ensure the quality of the beverage in the main distribution system regarding the concentration of these hazardous heavy metals. The concentration of common heavy metal contaminants on various malt beverage study areas is invited in this master's research project. The following significances are attributed to the study's findings.

- This study provided scientific explanation about the concentration of toxic heavy metals in malt beverage.
- For researcher who are interested in conducting study on malt beverage quality this work provide baseline information for further study on other heavy metal which are not included in this study .
- Gives evidence to the authorities for further improvement of the malt quality.
- To help the consumers make healthier choices.

1.8. Organization of the thesis

This research study is structured across five chapters. Chapter one encompasses the study's context, statement of the problem, and specific objectives, scope of the study, limitations, significance. Chapter two introduces the theoretical back ground of the study and literature reviews. Chapter three deal with experimental setup, sampling techniques, sample preparation and experimental measurement procedures. Analysis, findings, discussions of the experimental results, conclusions and recommendations are encapsulated in chapters four and five. In the reference section a material utilized in this thesis work was satiated as list of sources.

CHAPTER TWO

2. THEORETICAL BACKGRUOND

There are three sections to this chapter. I will cover some general optical spectroscopy, atom and interaction between light and EMR, atomic spectra, transition probability, selection rule and Einstein's coefficient in the first section. More focus is placed on atomic absorption spectroscopy in this section as an example of a spectroscopy. The second section features a presentation of quantification of heavy metals using AAS, malt drink and heavy metal contamination. This section provides specific information about heavy metals, their sources, and how they affect the human body. A review of some current literature that is relevant to this topic is done in the third section.

2.1. Optical spectroscopy

The study of light interaction with matter is called optical spectroscopy. It is used to determine the composition, structure, and dynamics of matter. Optical spectroscopy is based on the fact that When light interact with matter, it can be absorbed, scattered, or emitted. The amount of light that is absorbed, scattered, or emitted depends on the properties of the matter. By measuring the amount of light that is absorbed, scattered, or emitted, it is possible to determine the properties of the matter [17]. In other word, it is the study of the absorption and emission of light and other radiation by matter, which involves the splitting of electromagnetic radiation or light into its constituent wavelengths that is known as spectrum. More recently, the definition has been extended to include the study of the interactions between particles such as electrons, proteins, and ions, as well as their interaction with other particles as function of their collision energy. It was crucial in the development of the most fundamental theories in physics such as quantum mechanics, special and general theory of relativity, and quantum electrodynamics. The light wavelengths that a certain element specifically absorbs or emitted are used in the technique. They line up with the energy required to transfer an electron from one energy level to another enrgy level. [18]. Spectroscopy can be used to investigate differences in the kind of radiant energy used to interact with matter, the nature of the interaction, and the material type. The various parts

of spectroscopy that result from this interaction include molecular spectroscopy, emission spectroscopy, atomic absorption, microwave, infrared, electron, and other types of spectroscopy.

2.1.1. Electromagnetic radiation

Electromagnetic radiation is a sort of energy it will create when electrically charged particles flow through a vacuum or matter, there can be oscillation in electric and magnetic fields. The electric and magnetic forces moving perpendicular to one other while they are right angles to one another. Photons are released as electron radiation and quantization harmonic waves that travel at the speed of light. The electromagnetic spectrum is then used to classify this energy according to its wavelength. These waves, which are both electric and magnetic, have certain properties such as amplitude, wavelength, and frequency. The characteristic of electromagnetic radiation can be behaviors both as a wave and particle. The best way to describe some characteristics of electromagnetic radiation, including its diffraction, reflection, and refraction, is to think of light as a wave. Also considering light as a particle other characteristics like absorption, emission, and scattering are more accurately represented [19].

Wave-like properties

Light is considered wave-like in nature as it consists of oscillating electric (E) and magnetic (M) fields. These fields are at right angles to each other, and travel at a constant velocity in a given medium. In a vacuum, this velocity is 3×10^8 m/s. Numerous essential characteristics of an electromagnetic wave include its wavelength, polarization, phase angle, velocity, amplitude, frequency, and so on [20].

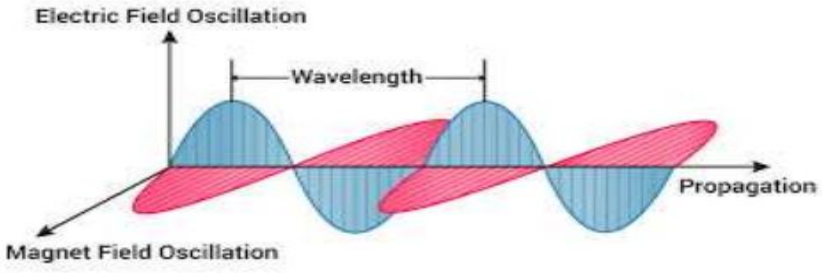

Figure 2.1: An EM radiation showing oscillator electric and magnetic field propagation

Particle-like properties

They are described in terms of energy packets known as photons. The energy associated with electromagnetic radiation can be defined as follows: E=hv, E is Energy (J), h is Planck's constant (6.62×10^{-34}Js), v is frequency (s^{-1}) or (Hz). In spectroscopy, wavelength is generally expressed in μm, nm, or wavenumbers, $\tilde{\nu}$ (where $\tilde{\nu}=1/\lambda$ and expressed in cm^{-1}). Electromagnetic radiation (EMR) consists of waves of the electromagnetic (EM) fields, which propagate through space and electromagnetic radiant energy. Types of EMR include radio waves, microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays, all of which are part of electromagnetic spectrum [20].

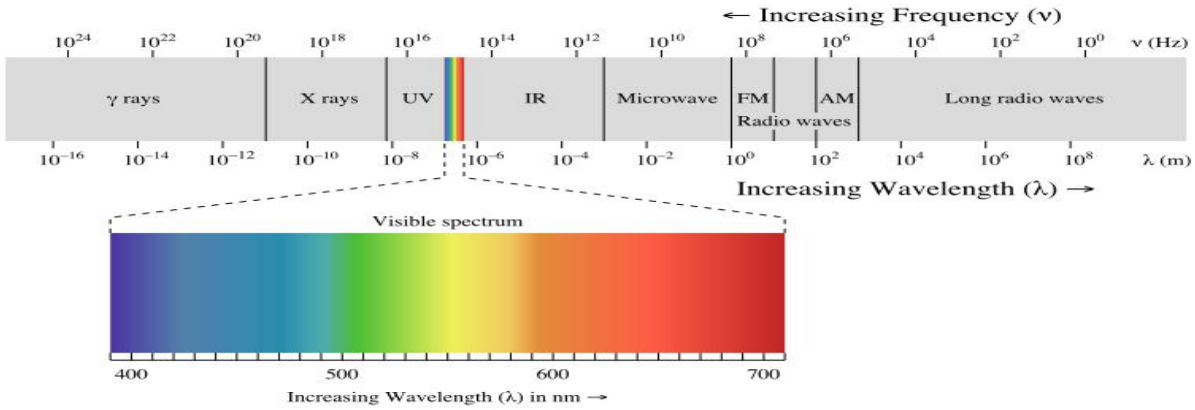

Figure 2.2: The region of electromagnetic spectrum [21].

2.1.2. LASER

Laser is a special kind of electromagnetic radiation that is produced though the principle of light matter interaction. Laser stands for Light Amplification by Stimulated Emission of Radiation. The laser device produces a coherent, powerful, monochromatic and collimated beam of light [22]. There are three components required for laser action the first one is active medium or gain medium which can support population inversion, the second one is an energy pump which is used to create population inversion in the active medium, and the third one is an optical resonator or cavity which is used to create high radiation density or amplification. Basics of complete processes in laser systems are first the gain medium is pumped by some methods; some atoms or molecules are excited. Following the excitation, spontaneous emission occurs in all directions but emission along axis of cavity is reflected back and forth through the gain medium. The

spontaneously emitted photons stimulate further emission from the medium and after that a large radiation density quickly builds up in the cavity. One of the mirrors is usually partially transmitting to allow some of the laser radiation to escape. Lasers are normally categorized as: gas, liquid, or solid state laser, depending on the nature of the active medium [22]. Gas laser are those lasers that uses gases as active medium. Example: He-Ne Laser, CO2 Laser, Argon ion laser, etc. Liquid or Dye lasers are those lasers where their active medium are liquid or usually dye solutions. Example: Rhodamine 6G, Coumarin dye laser, etc. Solid State laser are those lasers where their active medium is solid state materials. Example: Ruby Laser - Nd: YAG laser, Ti-Sapphire, semiconductor lasers (GaAs, GaAsP lasers), fiber lasers, etc.

2.2 Atoms

In terms of characteristics, an atom is the lowest unit of matter that is still an element. Electrons are said to occupy particular orbitals around the nucleus, and it is said to have discrete energy levels. Electron behavior surrounding nuclei is controlled by a number of quantum concepts, including quantum tunneling, quantization, uncertainty, wave-particle, and superposition. An electron can only exit in specific energy states within an atom due to quantization of its energy level. An atom is usually stimulated by laser light in laser spectroscopy, which results in electronic transitions between energy levels. From one energy level to another, or from one higher energy level to another energy level, an electron is transferred. According to the principles of quantum physics, an atom is a defined and little-studied entity. Atomic orbitals are those regions of space around the nucleus mostly to have electron in them. These orbitals, defined by mathematical function, are defined by quantum physics. An orbital is a representation of the probability distribution of finding an electron at specific location. Orbitals come in a variety of shapes and types, including s, p, d, and f orbitals. The spherical s orbitals orbit the nucleus. A p orbital resembles a dumbbell with its two lobes oriented in an x, y, and z configuration. Among the more complex geometries found in the d orbital are the dumbbell and cloverleaf shapes. The shapes of a f orbital are much more intricate. According to the Pauli Exclusion Principle, which stipulates that no two electrons in an atom may have the same set of quantum numbers, electrons occupy these orbitals [23]. The number of orbitals in energy levels is equal to square principal quantum number. Hence, energy level one will have 1 orbitals (1^2), energy level two have 4 orbitals (2^2) ,enrgy level three have 9 orbitals(3^3),energy level four have 16 orbitals(4^4). In quantum number (n, l ,m) n=1, 2,3….., l =0,1,2,3..(n-1) and m=-l, -l+1, 0, 1..,l-1, l . The ground

state (n=1) is denoted by 1s and 1st excited state (n=2) contain one 2s states and three 2p state, corresponding to m=-1, 0,+1 so total 4 states.

2.3. Interaction between light and atom

When light interact with matter (atom, molecules and ion) three processes can occur: absorption, stimulated emission, and spontaneous emission [24].

Absorption: If an atom in its ground state of energy E_1 and radiation of suitable energy ($h\nu = E_2 - E_1$) is give such that the atom goes to excited state E_2 i.e., its electron jumps from lower energy level E_1 to higher energy state E_2 by absorbing a quantum of radiation or photon. This kind of transition is called the absorption or induced absorption. This is shown in Fig.2.3

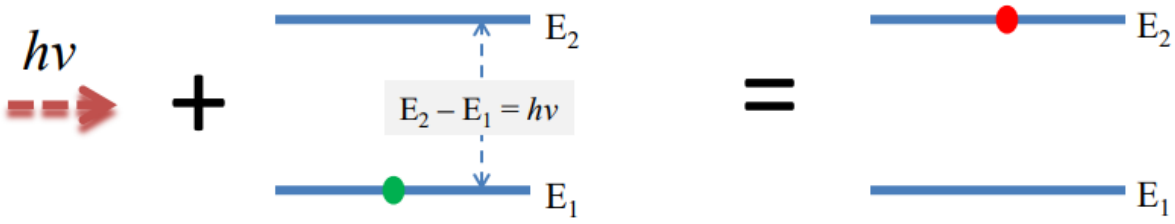

Figure 2.3: Absorption process of the atom interact with EMR

The probability per second that atom will be absorb a photon, $\frac{d(P_{12})absor}{dt}$ is proportional to the number of photons of energy hv per unit volume (spectral energy density, ρ (ν)).

$$\frac{d(P_{12})absor}{dt} = B_{12}\rho(\nu)$$

Here B_{12} is called the Einstein's B coefficient of absorption of radiation and ρ (ν) is the energy density of the radiation of frequency ν.

Stimulated emission: When an input photon with an appropriate energy ($h\nu = E_2 - E_1$) strikes an atom in its excited state (E_2), the atom (electron) may jump to a lower energy state (E_1) and produce another photon with the same frequency, same phase, and same direction. Thus, there are now two photons present, each with energy of $E_2 - E_1$. This kind of transition is called stimulated emission of radiation [24]. This is shown in Fig 2.4.

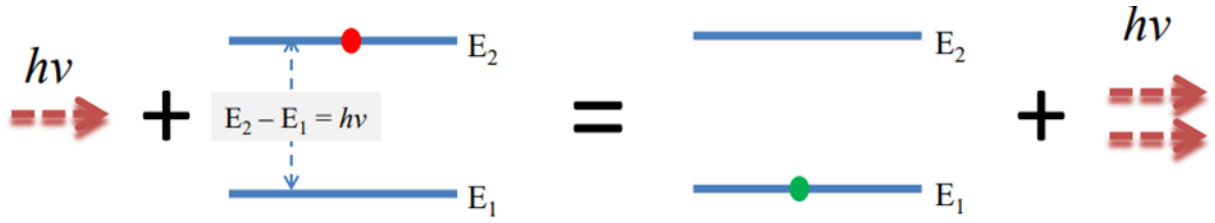

Figure 2.4: Stimulated emission process that atom interact with EMR

The probability per second that one emits one induced photon per seconds is:

$$\frac{d(P_{21})_{.stimu}}{dt} = B_{21}\rho(v)$$

Here B_{21} is called the Einstein's B coefficient of induced emission of radiation and ρ(ν) is the energy density of the radiation of frequency ν. Then the number of photons of energy hν emitted per second by induced emission in the system is equal to N_2 B_{21} ρ(ν).

Spontaneous emission: An atom can have different energy levels; the lowest energy level is the most stable state of an atom is the ground state (E_1) to move to the excited level (E_2), a higher energy level when it receives enough thermal or light (photon) energy. At this level, electrons are sometimes referred to excited atoms. Since an electron's life in the excited state is very short seconds (around10^{-8} seconds), after this period the electron returns to lower energy level E_1 by emitting photon. This process is called spontaneous emission. The frequency, v of the emitted photon is given by $v = \frac{E2-E1}{h}$. If there are large numbers of atoms in the upper energy level, then the emitted photon will have randomly different initial phases and directions and the emitted radiations will be incoherent. Figure: 2.5

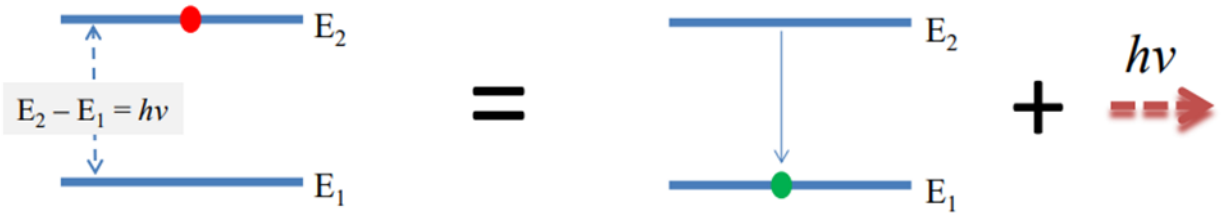

Figure 2.5: Spontaneous emission process atom interact with EMR

The probability per second that a photon is spontaneously emitted by an atom depends on electronic structure of the atom i.e. wave functions of the 1st and 2nd state but not on the external radiation field ρ (v).

$$\frac{d(P_{21})_{.spont}}{dt} = A_{21}$$

A_{21} is called the Einstein's A coefficient of spontaneous emission of radiation. Thus the number of photons of energy $E_2 - E_1$ emitted per second by spontaneous emission in the system is equal to N_2A_{21} [24].

2.4 Transition probability, Selection rules, and Einstein's coefficient

The value of the transition moment integral is used in spectroscopic selection rules in quantum mechanics, where μ is the transition moment operator and ψ_1 and ψ_2 are the wave functions of the two states that are engaged in the transition, states 1 and states 2, respectively. The like hood of a transition between states 1 and 2 is represented by the integral $\boldsymbol{D = \int \psi_1^* \mu \psi_2}$, and if its value is zero, the transition is forbidden. Through the transition moment D, the Einstein coefficients are associated with the wave functions ψ_1 and ψ_2 of the combined states, which can be expressed as $D = \int \psi_1^* \mu \psi_2$. The electric dipole moment operator for the interaction with the electric component of radiation is denoted by, which is defined as $\boldsymbol{\mu = \sum q\ r_i}$, where q denotes the charge and r_i denotes the particle's position vector. A dipole moment, represented by the Greek letter "μ", is the product of the charge magnitude and the separation [17]. Determining the symmetry of the transition moment function, D, suffices to provide a selection rule for the integral itself; further computation is not required. If the integral's value is non-zero and the transition is permitted, then an atom or molecules belong if the transition moment function is symmetric over the whole absolutely symmetric representation of the point group. The transition is prohibited otherwise. The transition moment function is anti -symmetric or odd if D is zero.

In the thermal equilibrium state (i.e., steady state) total number of photons absorbed per second should be equal to the total number of photons emitted per second. It can be written as

Total absorption rate = Total emission

$$N_1B_{12}\,\rho(\nu) = N_2\,B_{21}(\nu) + N_2A_{21}$$

$$\rho(\nu)\,[N_1B_{12} - N_2B_{21}] = N_2A_{21} \quad \text{divide both } N_2B_{21}$$

$$\rho(\nu) = \frac{A21}{B21}\left(\frac{1}{\frac{N1B12}{N2\,B21} - 1}\right) \ldots\ldots\ldots\ldots\ldots (1)$$

According to Maxwell-Boltzmann distribution the number of atoms N_1 and N_2 in the energy states E_1 and E_2, respectively, in the steady state at temperature T are given by

$$N_1 \propto e^{\frac{-Ek1}{T}} \text{ and } N_2 \propto e^{\frac{-Ek2}{T}} \quad \ldots\ldots\ldots\ldots(2)$$

Here k is the Boltzmann constant. Therefore

$$\frac{N1}{N2} = \frac{g2}{g1}\, e^{\left(\frac{E1-E2}{KT}\right)}$$

But $E_2 - E_1 = h\nu$ (energy of the photon emitted or absorbed).

$$\frac{N1}{N2} = \frac{g2}{g1}\, e^{\frac{h\nu}{KT}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Substituting the value of $\frac{N1}{N2}$ in Eqn. (1), we get

$$\rho(\nu) = \frac{\frac{A21}{B21}}{\frac{g2B12}{g1B21}(e^{\frac{h\nu}{KT}} - 1)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Planck derived an expression for $\rho(\nu)$ in the following form

Since $\rho(\nu) = \frac{8\pi\nu^2}{c^3}$, gives the number of modes per unit and frequency interval dv= 1Hz .

$$\rho(\nu) = \frac{8\pi\nu^2}{c^3}\left(\frac{h\nu}{e^{\frac{h\nu}{KT}} - 1}\right)$$

$$\rho(\nu) = \frac{8\pi h\nu^3}{c^3}\left(\frac{1}{e^{\frac{h\nu}{KT}} - 1}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Comparing Eqns. (4) and (5) for $\rho(\nu)$, we get $B_{12} = B_{21}$, $g_2=g_1$..... (6) and

$$\frac{\frac{A21}{B21}}{\frac{g2B12}{g1B21}(e^{\frac{hv}{KT}} - 1)} = \frac{8\pi hv^3}{c^3}\left(\frac{1}{e^{\frac{hv}{KT}} - 1}\right)$$

$$\frac{\boldsymbol{A21}}{\boldsymbol{B21}} = \frac{\boldsymbol{8\pi hv^3}}{\boldsymbol{c^3}} \text{ ..... (7)}$$

Equation (6) shows that transition probability of absorption is equal to transition probability of induced emission [25]. Relation (6) has also been proved in semi classical theory of radiation by using perturbation theory. Equation (7) shows that $\frac{A21}{B21}$ is proportional to ν^3. Also rate of spontaneous emission over dominate the stimulated emission and the probability of spontaneous emission dominates over stimulated emission and it increases more and more energy difference ($\Delta E = E2 - E1$) between the level increase.

This is the ratio of Einstein's A coefficient of spontaneous emission and Einstein's B coefficient of induced emission. The square of the magnitude of D is the transition probability and related to B_{12} is:

$$\mathbf{B_{12}} = \frac{\boldsymbol{8\pi 3}}{\boldsymbol{(4\pi\varepsilon 0)3h2}} \boldsymbol{|D|^2}.$$

The transition probability D^2 is related to selection rules in spectroscopy. $|D|^2 = 0$ for forbidden transitions $\neq 0$ for allowed transitions electric dipole selection rules [17]. The transition probability depend on the dipole moment of the atom between the lower and upper states.

2.5 Atomic spectra

When light is released at particular wavelengths, these transitions occur in the electrons of any element on the periodic table. The electromagnetic radiation generated or absorbed by an electron during these internal energy level transitions in an atom is represented by an atomic spectrum [26]. The frequencies of electromagnetic radiation emitted or absorbed by atoms are atomic spectra. Spectroscopy is the study of atomic spectra. To identify elements and molecules, and to study the structure of atoms and molecules are used atomic spectra. The Rydberg formula clearly split the hydrogen emission spectrum in to a number of wave length dependent spectral lines. The visible spectra lines in the hydrogen emission spectrum are cause by atomic transition

$$\nu = R(\frac{1}{n_1^2} - \frac{1}{n_2^2})$$

between distinct energy level. Estimates the wave length of the spectral line in a wide variety of chemical element in atomic physics for all atomic hydrogen transition, the equation is the generalization of Blamer series.

R=1.09737*$10^7 m^{-1}$(Rydberg constant), v = frequency, n=quantum number

Types of Atomic Spectra:

Emission Spectra: Light is released when a solid is heated to a high temperature or when a gas is exposed to an electrical discharge. It has been discovered that a spectrum known as the emission spectrum is formed. These occur when an electron transitions from a higher energy level to a lower one, emitting light of specific wavelengths, it corresponding to energy difference between the levels($h\nu_{ik} = E_k - E_i$). The resulting emission spectrum consists of sharp, discrete lines characteristic of the element. The spectra distribution of a radiant flux from a source . Example; Thermal radiation they are continuum distribution of an atom or molecules discreet emission spectra distribution (between two bound states).

Absorption Spectra: When electromagnetic radiation pass through a cool, dilute gas, atoms absorb photons with energies corresponding to the energy differences between the energy levels. This result in dark lines (absorption lines)in the continuous spectrum of the light source, indicating the wavelengths that have been absorbed by atoms in gas. The difference between incident and transmitted power of spectral distribution is called absorption spectrum. Bond level is discrete absorption spectrum, if E_2 is above dissociated limit (ionization energy) absorption spectrum become continuous. Absorption or emission spectral lines are never strictly be monochromatic, rather they will have spectral distribution around the central frequency $\nu_0 = \frac{Ek - Ei}{h}$.

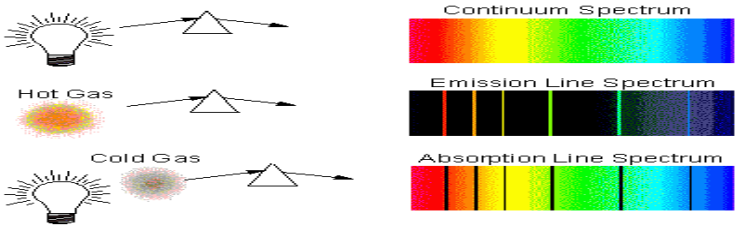

Figure 2.6: Emission spectra complement absorption spectra

2.6 Atomic spectroscopy

Atomic spectroscopy is studies the interaction between the EMR with the atom. A term used to describe an atom's normal and most stable orbital configuration is "ground state." An atom will absorb energy if it is given, and an outside electron will be encouraged to go into the "excited state," a less stable configuration [27]. The energy of the light absorbed is characteristic of the element, and the intensity of the absorption is proportional to the concentration of the element in the sample. It is a quantitative technique for measuring chemical quantities of element present in environmental samples such as water by measuring the absorption of light by atom in the free atomic state [28].

2.6.1. Atomic emission spectroscopy

In this method of chemical analysis, the intensity of light released by a flame or plasma excites an atom, causing the element to release a wavelength unique to it. The emission spectrum's wavelength of the atomic spectral line identifies the element, and the light's strength is directly proportional to the element's atom. There are various methods for stimulating the specimen [29]. ICP-OES is a type of atomic emission spectroscopy that is frequently utilized. ICP-OES is an emission spectroscopy method that uses plasma to excite atoms and ions to produce electromagnetic radiation with wavelengths distinctive of individual elements. It is an analytical technique for determining the amount of certain elements in a sample. The ability of atoms and ions to absorb energy allows electrons to migrate from their ground state to an excited state, which is how the ICP-OES principle works [30]. A group of atoms is exposed to light with a preset wavelength during the process of atomic absorption spectroscopy. A portion of the light is absorbed when the wavelength of the source light has energy equal to the difference between two atomic energy levels. The variation in light intensity gets absorbed. The discrepancy between the light source's (like a lamp) intensity and the value. The amount of a specific element (or atoms) in the sample can be ascertained using this absorbance value. The Beer-Lambert law provides the link between the concentration of atoms, the distance traveled through atoms in a collection, and the fraction of light absorbed [31]. Numerous applications exist for spectroscopy in various fields. Qualitative material analysis, the food business, forensic toxicology, astronomy, environmental

analysis, material technology, and the medical area are a few of the common uses for spectroscopy.

2.6.2 Atomic absorption spectroscopy

Atomic absorption spectroscopy is a technique that involves study of absorption of EMR in relationship to atomic structure. For absorption to occur, the energy differential between the higher and lower energy levels must be the same. The AAS makes use of the ability of an atomizer to produce free electrons and absorb radiation at particular frequencies. It measures how much ground atom absorption occurs in gaseous states. To put it another way, atomic absorption is technique used to quantify the concentration of metals. It involves the absorption of light at a wavelength specific to an element by free atoms in that element.

2.6.2.1 Working principle of AAS

When a sample of a particular atom is exposed to light of a specified wavelength, the atom or ion will absorption this light. All atoms and ions have the ability to absorb light at specific, unique wavelengths. The concentration of ions or atoms that absorb light at this wavelength directly correlates with the amount of light absorbed. An atom's electrons can excite at different energies. The photon energy is absorbed by the atom and electrons transition from ground state to excited state when it is subjected to radiation with a wavelength that is specific to it. The transformation that takes place during this phase is closely tied to the electron's ability to absorb radiant energy. Additionally, since every element has a distinct electrical structure. It is possible to measure the radiation absorbed, which is a characteristic specific to every single element. Utilizing these fundamental concepts in real-world quantitative analysis is what an atomic absorption spectrometer does [32]. The Beer Lambert law describes the relationship between light absorption and concentration of the element. According to the law, the amount of light absorbed is proportional to the number of atoms excited from the ground state.

2.6.2.2 Quantitative analysis of AAS results using Beer lambert law

When a beam of light passes through a solution containing a light-absorbing an analyte, this states is the Beer-Lambert law the relationship between the analyte concentration and the absorption is described by: $A = - \log 10 \left(\frac{I}{Io}\right) = \varepsilon bc$ Where A is the absorbance, Io the light beam radiant power before attenuation, I the attenuated radiant power, ε the molar absorptivity, b the path length through the absorbing medium and c the analyze concentration. The Beer-Lambert

law can be split into two parts with Beer's law stating that there is a linear relationship between absorbance and concentration at a fixed path length, and Lambert's law stating that there is a linear relationship between absorbance and path length at a fixed concentration [33]. The basic principle of beer lambert is common to atomic absorption and atomic emission. The grater the concentration there is the greater the absorption or emission.

2.6.2.3 Basic Instrumentation of AAS

The standard AAS instrument consist four component

1. Light Source - Hollow Cathode Lamp(HCL)
2. The sample introduction area, atomizer(nebulizer, mix chamber ,flame)
3. The monochromator
4. A detector

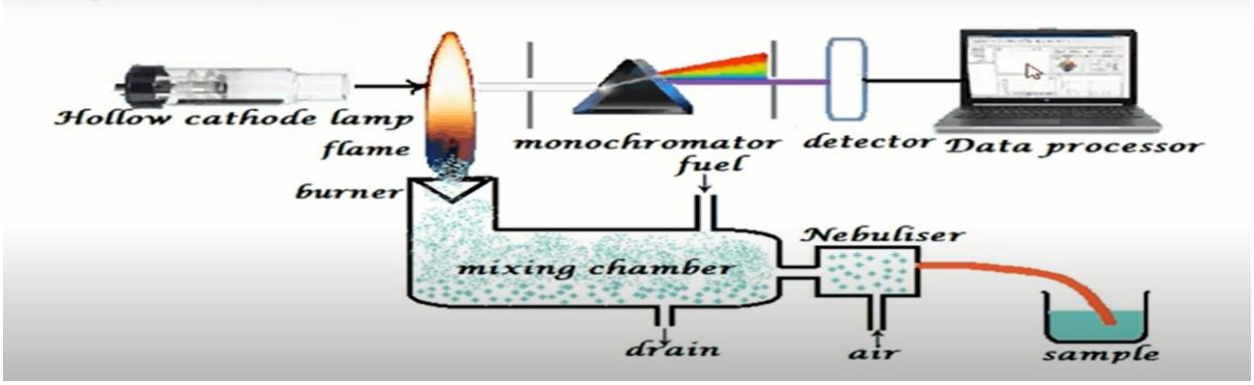

Figure2.7: Diagram for the working component of FAAS

<u>Light Source (HCL)</u>

Light is absorbed by atoms at specific wavelengths. To achieve the highest level of sensitivity in measuring this narrow absorption of light, a line source that emits the precise wavelengths that an atom may absorb must be used. The HCL is a line source or radiation source that is used to elevate the energy level of the target metal's electrons and produce a steady, powerful output. In atomic absorption spectroscopy the one kind of discharge lamp utilized as a light source is a hollow cathode lamp. It is made up of a glass tube that is cylindrical and holds an anode, cathode, and a little quantity of an inert gas, such as argon. An air tight neon or argon lamp

operating at 1 to 5 torr. As a high voltage potential difference is produced between the tungsten anodes, inert gas is ionized. A plasma discharge forms when a highly voltage is delivered between the anode and cathode, ionizing the gas. When exposed to electron's the cathode material produces distinctive spectral lines that provide the precise wavelengths required for elemental analysis in AAS [34]. Due to this ionization of inert gas take place and ions are produced. Positive ions are attracted towards negative electrode (Cathode) and strike on the surface of cathode. As a result of this atom from the surface of the cathode is ejected, this process is a called sputtering.

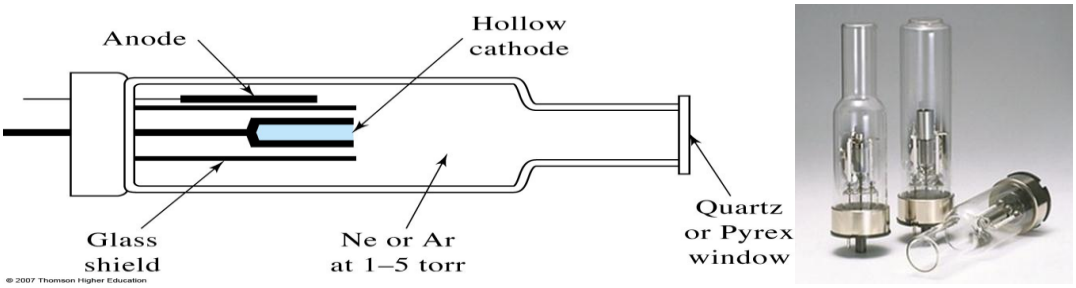

Figure 2.8: A hollow cathode lamp light source.

Atomization: FAAS, GFAAS, CAAS

The technique of atomizing a sample for atomic spectroscopy analysis involves breaking the sample up into individual atoms. Depending on the kind of spectroscopy being employed, such techniques as flame atomization, electro thermal atomization, or cold vapor atomization can be used to accomplish this [35]. Burners and nebulizers make up the flame atomizers. The purpose of the nebulizer is to turn the fluid into an aerosol or fine mist. A flame is used in a flame atomizer to carry out atomization. The metallic element undergoes optimization and regulated thermal energy conversion to atomic dissociated vapor. In order to create a flame, fuel and oxidant gasses are delivered into the mixing camber through the nebulizer's reverse peristaltic suckering action, which travels to the spray chamber and ultimately to the burner head. Only tiny droplets that are known as aerosols travel from spray chamber to the burner head. Preferred and cautious readjustment of the gas is to be done since the larger droplets are refined out through the ejected out let of the nebulizer in the narrow burner [36]. In CVAAS, the atomization process involves converting the analyte into its gaseous states without thermal decomposition. This is achieved by using a reducing agent, usually stannous chloride ($SnCl_2$), to generate volatile species of the

element of interest. The volatile species are then transported to the atomizer, where they are atomized [28]. GFAAS, the sample is introduced to the graphite Furnance, where it is dried and ashen at low temperature to remove any volatile component. The temperature of the graphite is rapidly increased to a temperature where the analyte undergoes atomization. This temperature is specific to each analyte and is typically in the range of 2000ºC to 3000ºC. During atomization, the analyte is converted in to free atoms in the gas phase.

Monochromator:

An optical tool called a monochromator is used to choose a limited range of wavelengths from a larger spectrum of light. It is composed of a dispersive element (such a prism or diffraction grating) that divides light into its component wavelengths so that only the appropriate wavelength can pass through [37]. Light with a single wavelength conversion is known as monochromator light. These electromagnetic wave radiations only have one wavelength, which gives the impression of one single hue to the eye. HCL emits a spectrum of lines, which a monochromator separates into a single atomic resonance line. Fundamentally, it is a movable filter that isolates all wavelengths outside of a predetermined, restricted spectrum region and chooses one for transmission to the detector. Entrance silt, a dispersion device, and an exit silt make up a monochromator.

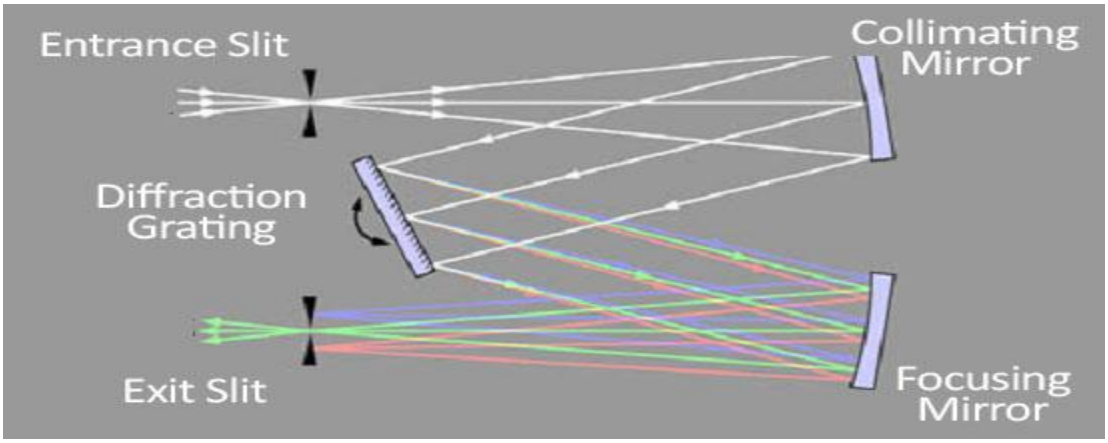

Figure2. 9: Model of a monochromator used by an AAS machines

There are two different ways to create monochromatic light: prisms and grating systems. Materials with a lot of parallel, tightly spaced ridges or slits are used in diffraction gratings. For

every wavelength, diffraction produces constructive interference at different sites. A concave mirror is used to collimate the split wavelengths in the direction of the exit silt. The grating device's tilt angle establishes the wavelength band that leaves the monochromator and enters the detector. The selection of photons according to the wavelength that will reach the detector is done by the monochromator [38].

Detector

A monochromator light can be converted by a detector into a more straightforward electrical signal. The charge-coupled device (CCD) detector in the atomic absorption spectroscopy instrument has largely replaced the photomultipliers used in older equipment for detecting purposes. One can adjust a detector such that it responds at a certain wavelength or frequency.

Recorder

The detector can send electrical signals that the recorder can receive and process into a response that can be read. Today, we employed an appropriate computer system with software to recode data from the detector in the instrumentation used for atomic absorption spectroscopy.

2.7 Quantification of heavy metal using AAS

2.7.1 Heavy metal

Heavy metal is the metallic elements with a density that is higher than that of water. Metalloids like arsenic, which can be harmful at low exposure levels, are also considered heavy metals. Heavy metals are typically utilized in paint as additives and pigments. Both directly and indirectly, humans are at risk from the presence of certain heavy metals [39]. On the other hand, heavy metals like lead, nickel, chromium, and cadmium are known to offer significant health risks to humans. They are also able to induce impairment and dysfunction of biological pathways for the blood, colon, liver, kidney, skin and cardiovascular system [40].Their difficult to break or destroy nature; heavy metals are naturally occurring components of the earth's crust and a continuous source of pollution [41]. The cause for the heavy metal contamination are the natural (rock) and anthropological source (metal mining, smelting, trash dumping, incineration, pesticides, et...)[42] . Heavy metal contamination of food and drink is occurring due to pollution of

the land, atmosphere, subsurface and surface waters [16]. Arsenic, lead, and cadmium must be measured in food and drink due to their high toxicity [17].

Quantification of heavy metals using AAS is common analytical technique that was employed in beverage. AAS is a technique used to measure the concentration of an element in a sample by measuring the amount of light absorbed by the element when it is excited by a specific wavelength of light. It is sensitive and accurate technique that can be used to measure the concentration of a wide range of the elements, including heavy metal such as, Co, Pb, Cu, and Cd. The following steps are involved in quantifying heavy metals using AAS; Prepare the sample by dissolving it in a suitable solvent. According to the method of Wallace, sample preparation prior to analysis, the samples were it digested [43]. To 25ml of sample in a 100ml beaker, add 1ml 30% hydrogen peroxide and 100ul of concentrated nitric acid and place sample on hot plate. Digests for a few half an hour, remove the beaker and allow it to cool temperature. Add 2.5ml of 1% ammonium phosphate and dilute to 25ml. Transfer the solution to a 30ml plastic bottle [44]. The calibration plot method described in British Pharmacopoeia was adopted for the preparation of metal ion and AAS analysis [45]. A stock standard solution, by dividing the molar mass of the compound contains the element of the meal ions was prepared by the molar mass of the element. The weigh obtain was equivalent to 1.0g of the metal ion. This weight was dissolved in 100ml to give 1000ppm. The absorbance of the solution was obtained using AAS for each heavy metal. The calibration graph was plotted and the regression equation was determining the heavy metal concentration. Measure such as blank determination, spike recovery and instrument calibration checks are performed to ensure the accuracy and precision of the result. In soft drink is based on two units of measurements; the maximum contaminant level goal (MCLG) and maximum contaminant level (MCL), United States Environmental protection agency defined as the standard for the determination of the heavy metal contamination. MCLG is the level of a contaminant in drinking water below which there is no known or expected risk to health and hence allow for a margin of safety and are non-enforceable public health goals. On the other hand, the highest level of contaminant that is allowed in drinking water is MCL. The MCLG and MCL are measured in milligrams per liter (mg/L) which is equivalent to pat per million [46]. AAS plays a crucial role in ensuring human and environmental safety by detecting and quantifying heavy metals. To quantify heavy metal has to be the maximum permissible limit that was set by international standards like WHO, BIS, USEPA [47].

Table 2.1: Guideline values of heavy metals in drinking water.

Metal Name	USEPA (maximum contaminated level)	WHO Guidelines	BIS(permissible limit)	Ethiopia standard	Source
Lead(Pb)	0.015	0.01	0.01	0.01	(WHO,2011)
Chromium(Cr)	0.1	0.05	0.05	0.05	(WHO,2011)
Cobalt(Co)	0.05	0.01	0.05	0.05	(WHO,2011)
Nickel(Ni)	ND	0.02	0.02	0.02	(WHO,2011)
Copper(Cu)	1.3	2.0	1.5	1.0	(WHO,2011)

2.7.2 Malt drink and heavy metals contamination

Malt drinks are rich in vitamins and other nutrients, it was often given to sick and a young person as sustenance, but it has now become a popular beverage for individuals of all ages [3]. Some health advantages of the beverage include its ability to prevent ulcers, cancer, and coronary heart disease [4]. Malt beverages are often made with barley malt, though they can also be made with wheat, oats, rye, and sorghum in certain situations [5]. In order to make malt drinks, wort granules are typically dissolved in water, filtered, pure hop fragrance is added, and carbonation is then implemented. In addition to its delightful flavor, malt also offers vitamins, minerals, fiber, phosphates, antioxidants, and a vital means of hydration but it contains some heavy metal. Malt is used in the production of distilled spirits, alcoholic and non-alcoholic beverages, porridges, gruels, and foods for weaning [48]. Brewing enzymes are obtained from the malts; these enzymes are mostly carbohydrases (especially amylases, which hydrolyze starch into fermentable sugars) and proteases (which hydrolyze proteins into soluble short peptides and amino acids that the culture yeast uses) [49]. Malt drinks without alcohol, such as Guinness and malt-based beverages, have become more popular over time due to their increased nutritional value [50]. Soft drinks (malt beverages) have the potential to include harmful substances due to contamination of ingredients, surface water, and groundwater utilized in production. Manufacturers have also

stated that contamination occurs during the beverage processing, packaging, and storage stages [51, 52]. The production of beverages typically employs plant material as an active ingredient. For instance, during the production of beers, hops and cereals are used, which are plant-based and the soil in which they are cultivated could be contaminated by pesticides and fertilizers, which may contain traces of heavy metal [53].

2.8 Reviews of related studies employing AAS technique on different drinks

Some of the related research works performed previously by different researchers are reviewed below.

Woyessa, *et al* (2015) use flame atomic absorption spectrometry (FAAS) to determine the amounts of nickel, cadmium, chromium, lead, manganese, and copper in six popular soft drink brands in Harar, Ethiopia. There is a significant concentration difference ($p = 0.05$) in the mineral contents among the six soft drinks based on the FAAS results. Pb, Cr, and Cu concentrations were found to be significantly greater in Pepsi Cola, Mirinda, and Ambo flavor soft drinks than in Mn, Ni, and Cd concentrations. In contrast, although the amounts of Ni and Cd are within the acceptable range of national and international regulations, the level of concentrations was lower**.** Overall, according to the experimental results, all soft drink samples had Ni, Cd, Pb, Mn, and Cu levels that were within permissible bounds. Among all the components examined, there were statistically significant differences in the measured concentrations of Cr for each of the six soft drink groups in this study ($p = 0.05$). Thus, it was recommended that health authorities and soft drink manufacturers focus more on where these metals come from when making their products [54].

Oladapo, *et al* (2018) study a growing trend of alcoholic drinks flavored with what are thought to be botanical extracts having therapeutic properties. There is little information available about these items' phytochemical and heavy metal compositions. In this study, major motor parks in Ibadan, Nigeria's phytochemical characteristics and heavy metal concentrations of alcoholic beverages flavored with herbal extracts were evaluated. Standard techniques were used to analyze the phytochemical properties of the beverages in triplicate, and atomic absorption spectrophotometry was employed to evaluate the heavy metal levels. ANOVA was used to compare means at $p < 0.05$ after descriptive statistics were used to evaluate the data. The drinks had an alcohol concentration of 34.0–51.5% and a pH range of 3.28–6.57 %. Detected significant

phytochemicals and concentration ranges were phytic acid (0.72–2.37 mg/g), alkaloids (0.42–4.11 mg/g), flavonoids (0.22–3.64 mg rutin equivalents/g), total phenols (1.13–3.66 mg gallic acid equivalents/g), anthraquinones (0.74–1.93 mg/g),and triterpenoids (0.74–1.93 mg/g). Pb (2.13–4.70 mg/L), Cd (0.06–0.07 mg/L), Co (0.12–0.23 mg/L), Zn (0.14–0.40 mg/L), and Fe (0.72–4.22 mg/L) were the heavy metal levels; only Pb and Cd exceeded the World Health Organization's (WHO) waterborne limits of 0.01 mg/L and 0.03 mg/L, each. Beneficial phytochemicals and trace amounts of heavy metals can be found in the alcoholic beverages flavored with herbal extracts. Public health would benefit from customers being aware of the safety of these items in order to improve their health [55].

Oyekunlee, *et al* (2019) studies the levels of As, Cd, Co, Cu, Mn, Pb, and Zn in a few widely consumed soft drinks with varied packaging was examined. This was done to assess the impact of the materials used for the containers on the amounts of potentially harmful metals in the drinks as well as the short- and long-term health effects of regular use of these items. Bulk purchases of variously packaged soft drinks (in glass, can, and plastic bottles) were made in Ile-Ife, Nigeria, at wholesale retailers. To calculate the total metal content of the acid-digested samples, an atomic absorption spectrophotometer (AAS) was employed. The majority of the potentially hazardous elements examined in the study had quantities over the USEPA and WHO permitted limits, according to the report. At a 95% confidence level, there was a substantial impact of the packaging materials on the content of certain metals that were evaluated in the soft drinks. The study's findings suggest that since regular and consistent intake of these soft drinks may represent a substantial risk of exposure to potentially harmful metals, care should be taken when doing so [56].

Godwill, *et al* (2015) assessed some of the ingredients in 26 soft drinks sold in Nigeria and looked into the possibility of heavy metal contamination. Nigerians use soft drinks on a regular basis because of their taste, affordability, and ability to slake thirst. Nonetheless, the increased demand can jeopardize the production quality due to heavy metal pollution, which has been linked to human intoxication and mortality. The soft drinks were screened to check for alcohol, carbon dioxide, sugar, and phosphate. The pH and acidity were also measured. An atomic absorption spectrophotometer was used to measure the levels of lead, mercury, and cadmium. Alcohol, phosphate, carbon dioxide, and sugar were all found in the soft drinks, according to the

study. With a mean pH of 3.6 and an acid concentration of 8.1 g/L, the soft drinks were naturally acidic, with a pH range of 3 to 5 and a comparatively low acid concentration. Lead was detected in every sample, with a mean of 0.8 and a range of 0.17 to 3.39 mg/L; mercury was found in 22 samples, with a mean of 2.08 mg/L and a range of 0.29 to 11.32 mg/L; and cadmium was found in only one sample, with a mean of 0.149 mg/L. The majority of the samples had levels of heavy metal pollutants beyond the allowable threshold for high-quality drinking water when compared to EPA, WHO, and NIS guidelines. These findings imply that heavy metal contamination of soft drinks may exist in Nigeria, which poses a serious risk to the public's health. Quality control is therefore advised throughout the production process, particularly during the sterilization and purification phases [57].

Bunu, *et al* (2022) assessed the potential health concerns associated with the five heavy metals found in a selection of Nigerian soft drinks. Zinc, magnesium, aluminum, lead, and arsenic concentrations were measured using atomic absorption spectroscopy (AAS). All around the world, soft drinks are widely consumed. Owing to excessive demand, legal requirements might be broken, which could result in heavy metal contamination. There were twelve (12) soft drinks (designated B1–B12) utilized. After the AAS, parameters such as target health quotient (THQ), average daily intake (EDI), and chronic daily intake (CDI) were evaluated. The level of arsenic found in all soft drinks ranged from 0.001- 0.0603 mg/L, as against the 0.01 mg/L maximum contaminant level (MCL) standard. Lead was 0.001- 0.023mg/L (MCL is 0.01 mg/L) and within the MCL except in one sample (0.023 mg/L). Aluminum levels ranged from 0.001-2.0491 mg/L except in one sample (0.001 mg/L) and the WHO limit is 0.02 mg/L. The concentration range for magnesium was 0.6954-2.4341 mg/L versus the standard limit of 0.02 mg/L. The THQ values of aluminum were significantly different from the values of other heavy metals. The order of abundance of the metals was: Mg > Zn > Al > Pb > As. In comparison to the other metals, arsenic and lead were discovered in comparatively low amounts. In the majority of the samples, the aluminum THQ value was greater than usual. In all of the soft drinks, the magnesium content exceeded permissible limits. With the exception of aluminum, all heavy metals were under permissible THQ ranges. The necessity of quality control procedures during manufacture is highlighted by the high values of magnesium and aluminum [58].

Ogunlana *et al* (2015) Reports have indicated that heavy metal concentrations in Nigerian food and drink products above the WHO's recommended threshold. In this study, the levels of zinc, copper, lead, iron, and arising in a selection of Nigerian soft drinks were compared to the WHO's recommended limits. Nigeria has chosen this heavy metal. After being broken down by nitric acid, soft drinks were measured by the Alpha Omega Society. 30% of the chosen drink passed WHO standards for the heavy metal examined, according to the results, while the remaining 70% had metal content that was more than the WHO's maximum limit [59].

Salako *et al* (2015) the study was conducted at the popular border town of Idiroko in Ogun, Southwest Nigeria, which is a gateway for many ECOWAS countries and is well-known for the transportation of illicit products and services. The expansion of canned alcoholic and non-alcoholic drinks was the focus of the study, which also identified several of their properties that should be of public health concern. Therefore, a total of 28 canned alcoholic (Harp, 33, Star, Gulder, Guinness, Heinken, Turbo, Smirnoff and Red Bull) and 31 canned non-alcoholic (Farouz, Snappes, Cocacola, Sprite, Amstel Malta, Fanta, Malta Guinness and Maltina) samples were collected from Idiroko border area of Ogun State, Nigeria to determine levels of essential and toxic metals detected in these consumed products. The numbers of samples as shown in the methodology were prepared with standard wet digestion procedure, while the metals were analysed using the Atomic Absorption Spectrophotometer (Buck 210) techniques and the results were then compared with WHO standards : The metal concentrations ranges from 0-3.25 mg/l, 20.08-133.20 mg/l, 0.97-2.45 mg/l, 0- 0.44 mg/l, 0-0.26 mg/l, 0-0.30 mg/l, 0-0.14 mg/l, 0.11-7.38 mg/l, 0-0.02 mg/l for Cu, Mg, Fe, Pb, Cd, Ni, As, Zn, and Cr respectively. High concentration of toxic metals such as Pb, Cd, and Ni above WHO recommended limit were recorded from some non- alcoholic samples but all the alcoholic samples revealed low concentration of Cd. The samples were rich in essential metals such as Zn, Mg and Fe. To prevent instances of health risk due to ingestion of toxic metals, the proliferation of alcoholic and non-alcoholic drinks through the border must be controlled. The study recommended that port health services and security personnel be strict in maintaining the inflow of standard and non-expiry goods [60].

CHAPTER THREE

3. MATERIALS AND METHODS

3 .1 Instruments and apparatus

Measuring cylinder was used to measure the required amount malt beverage sample. Digestion flasks were used during sample digestion. Volumetric flask was used to measure and prepared intermediate standard solution. Plastic bottle of 100ml volumes were used to dilute sample solution and prepare standard solution. In the measurement and preparation of sample and standard solutions of plastic beakers, measuring cylinder and pipette were used. FAAS (BUCK SCIENTIFIC. AG250 AAS, USA) was used for determination of Cr, Pb, Co, Cu, and Ni content in malt beverage.

3.2 Reagents and chemicals

The following analytical grade chemical were use as received stock standard solution of 1000 mg/L, in 2 % HNO_3 of the heavy metals Cr, Cu, Co, Pb and Ni, (Buck Scientific Puro- Graphic) were used for preparation of intermediate standards. Working standard solutions for the construction of calibration curves were prepared by appropriate dilution of intermediate standard solutions of 1000 mg/L of the metals Cr, Cu, Co, Pb and Ni, in HNO_3 with de ionized water. Nitric acid was used for digestion of samples. De ionized water was used throughout this work.

3.3 Methods and experimental works

3.3.1. Experimental site

All experimental work was completed at Hawassa University's Analytical Chemistry Laboratory, including sample preparation and digestion, stock solution preparation, and AAS determination of heavy metal concentrations.

3.3.2 Sample collection

In the present investigation, five different brands of malt drinks such as: Malta Guinness, Sofi Malt, Nigus Malt, Bertat Malt, Sinq Malt samples were collected from Hawassa supermarkets and groceries to serve as the subject of study. Malt drink samples will be randomly selected and stored under room temperature in laboratory until they were required for analysis.

3.3.3 Cleaning the materials

To remove any heavy metals from its surfaces, 10% concentrated nitric acid (HNO_3) was used to clean all glassware. Volumetric flasks, measuring cylinders, digestive flasks, plastic beakers, and bottles were among the apparatus that underwent a thorough detergent wash before being rinsed with deionized water. The equipment was soaked and cleaned in deionized water before each usage. Disposables and tap water were used to clean equipment such the measuring cylinder and digestion flasks, which were subsequently rinsed with deionized water.

3.3.4 Sample treatment

To facilitate the digestion of the 25 ml malt drink sample, 10 ml of 5M nitric acid was added. According to the equation, the additional of nitric acid to remove organic materials by decomposing into carbon dioxide (CO2) and covert the metal present into soluble forms .

$$(CH_2)n + 2HNO_3 \rightarrow nCO_2 + 2NO + 2H_2O$$

In a fume cupboard, the mixture was evaporated on a hot plate until the brown vapors vanished and were replaced with white fumes. 50 ml of distilled water were added, and this was reduced to 25 ml by evaporation on a hot plate. To make 50 ml, 25 ml of deionized water were then added. At that point, it was ready for the AAS.

3.3.5 Standard solution preparation

A Stock solution of selected heavy metal (Ni, Cr, Pb, Co and Cu) of 1000mg/L was purchased from Central Laboratories of Hawassa University. The stock solutions were used for preparation of four intermediate standard solutions for calibration of the instrument before the direct measured concentration of malt beverage samples. Serial dilutions were made to prepare a series of standard solution from a stock solution of each metal. The expected sample concentrations are within the range established by the standard. These standards prepared by dilution from 1000mg/L stock solution were as follows: 0.05 mg/L, 0.1 mg/L, 0.5 mg/L and 1 mg/L for Nickel, 0.05 mg/L, 0.5 mg/L, 1 mg/L and 2 mg/L for Chromium, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L and 1 mg/L for Lead, 0.05 mg/L, 0.5 mg/L, 0.1 mg/L and 2 mg/L for Cobalt, 0.01 mg/L, 0.05 mg/L, 0.5 mg/L and 1 mg/L for Copper. Calibration curves were drawn for Ni, Cr, Pb, Co and Cu by plotting absorbance versus metal ion concentration.

3.3.6 Heavy metal determination on digested malt samples by FAAS

For the determination of heavy metals in the samples, the equipment's and instruments used in this study were calibrated to check their status before and in the middle of the experiments. Four series of working standard solutions were made from 10 mg/L intermediate standard solutions of the corresponding metals, which were made by diluting the stock standard solutions of the metals with de-ionized water, in order to determine the presence of heavy metal in a selection of malt beverage samples. These were carried out since a great deal of analytical techniques need for instrument calibration, which is a crucial step in the majority of measurement processes. The concentration of heavy metals in the samples and solutions was measured directly by FAAS by adjusting the light beam's wavelength to match each of the necessary elements in turn. As soon as the instrument was calibrated, the test arrangements and the reagent clear were progressively sucked into the nuclear retention spectrophotometer. For each test arrangement and reagent clear arrangement, at least three readings were obtained. The concentration of follow metals in reagent clear and tests arrangements were measured utilizing instrumental working parameters given in Table 3.1;

Table 3.1: Instrumental operating conditions for determination of Cu, Cr, Co, Pb and Ni heavy metals using FAAS

Metal	Wave length(nm)	Silt width(nm)	Lamp current(mA)	Energy (ev)	Instrumental detection limit(mg/L)
Cr	357.9	0.7	2.0	2.712	0.040
Ni	341.5	0.2	7.0	2.624	0.020
Cu	324.7	0.7	1.5	3.938	0.005
Pb	283.2	0.7	2.0	2.874	0.040
Co	240.7	0.2	4.5	3.106	0.050

FAAS (BUCK SCIENTIFIC MODEL 210VGP AAS,USA)were prepared with deuterium bend back ground corrector and standard discuss acetylene fire framework utilizing outside calibration bend after the parameters (burner and light arrangement, opening width and wave length alteration) were optimized for least flag concentrated of the instrument. Empty cathode lights worked at the producers suggested conditions were utilized at their particular essential source line. To ensure suitable fire conditions, the stream rates and acetylene were monitored. Metals were selected based on concentration mode, and each test arrangement's instrument readout was physically recorded. To ensure that the components were arranged clearly, the same explanatory technique was applied.

3.4 Validation of the procedure

3.4.1 Accuracy and precision

It is difficult to precisely assess the accuracy of the reading value since we do not know the specific concentration of heavy metals in the study samples. Therefore, in order to approximate the accuracy of the reading, spiking experimentation was employed. The accuracy of the data was evaluated using the pooled standard deviations and relative standard deviations of the triplicate sample measurement of specific solution and triplicated reading for each sample solution [61]. The precision of the results determined in this work also described by the standard deviation.

3.4.1.1 Spiking experiment

Spiking method on the same amount of sample of Sinq malt used for FAAS analysis of heavy metal was adopted to determine the reliability of the method. Because of the FAAS response signal is the same for analysis of any sample solution only a Sinq malt sample was selected for spiking experiment. 0.10 mg/L of Co, 0.10 mg/L of Cr, 0.10 mg/L of Ni, 0.10 mg/L of Cu and 0.10 mg/L of Pb were used to spike 25ml of Sinq malt sample at once in a round bottomed flask and then the sample were digested as used for original sample. Then the digested spiked sample were transferred in to a 100 mL plastic bottle and diluted up to the mark with de ionized water. Finally the solutions were analyzed for each element with atomic absorption spectrophotometer. As used for original samples triplicate spiked samples were prepared and triplicate readings were recorded. The original spiked samples concentration of five metals reported were determined from the concentration of diluted spiked samples and regent blank solution similar to the

concentration of heavy metal in original malt sample. Finally the accuracy of the result in this work checked by the efficiency of the optimized procedure based on recovery experiments. The percent recovery test of the total method used through the work was calculated using the following formula for each of five heavy metals studied.

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

Linearity method: is determining the stock solution of heavy metal using the solvent at five different concentrations by constructing calibration curve. Intermediate standard solutions (10 mg/L) of metals of interest were prepared from the 1000 mg/L standard stock solutions. These solutions were diluted to the desired concentrations to calibrate the instrument (A 10 ppm multi-element solution containing Cr, Cu, Co, Ni and Pb, was utilized to prepare elemental calibration solutions). Then the calibration curve was established according to the obtained response and the concentrations of heavy metals in standard solutions. The results show a good linear relationship.

$$y = a + b * x$$

Eg of the Ni heavy metal, $y = 0.0003+0.00272x$

$$R^2=0.9999$$

Method of detection limit (MOD): the expressed as concentration (amount) is derived from the smallest measurement that can be detected by single measurement with reasonable certainly for a given analytical procedure. It determine from reading three of solution where prepared following the same procedure as the samples. In this work for each samples of three solution concentration was determined for the elemental of the matter (Cr, Co, Ni, Pb, and Cu) by FAAS. The MOD is based on the standard deviation of replicated analyses (n=9 or more) typical sample estimated detection limit. The MOD was calculated as ($MOD = \sigma \times n$) where n=9.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1Calibration curve of the standard solution

Analyzing a measured quantity against a reference value is the process of calibrating an instrument or piece of technical apparatus. Intermediate standard solutions of 10 mg/L were prepared in a volumetric flask in this investigation using atomic absorption spectroscopy standard solutions comprising 1000 mg/L. Four series of working standard solutions of known concentration were prepared for five heavy metals. The corresponding absorbance of each series of working standard solutions were measured by FAAS as depicted in Table 4.1 with the known concentration of working standard solution. The absorbance of each five heavy metal was measured by FAAS at their specific wave length. The measurements procedure was done in triplicated for each solution of known concentration solution. The calibration curve for each selected heavy metal to be analyzed was prepared by plotting the absorbance as a function of heavy metal concentration of a series of standard solution. The correlation coefficient square, also known as the coefficient of determination, is a measurement employed in this work to indicate the extent to which the concentration variability of a solution influenced the corresponding absorbance variability. For five heavy metals, the correlation coefficient square was found in the range of 0.9988 to 0.999. This demonstrates that there is a strong positive association between each of the five heavy metals concentrations and the related light absorption.

Table 4.1:-Working standard concentrations of Ni, Cr, Pb, Co, Cu and the correlation coefficient

Element	No	Concentration of working standards(mg/L)	Absorbance	Correlation Coefficient (R^2)
Ni	1	0.05	0.001751	
	2	0.1	0.00287	
	3	0.5	0.01381	0.9999
	4	1	0.02748	
Cr	1	0.05	0.004185	
	2	0.5	0.03992	0.9999
	3	1	0.076514	
	4	2	0.154121	
Pb	1	0.05	0.001168	
	2	0.1	0.02418	
	3	0.5	0.01301	0.9993
	4	1	0.027568	
Co	1	0.05	0.00108	
	2	0.5	0.02258	0.9988
	3	1	0.044329	
	4	2	0.085107	
Cu	1	0.01	0.001247	
	2	0.05	0.009121	0.9994
	3	0.5	0.078724	
	4	1	0.164248	

The regression linear plotting a functional standard concentration curve using ORIGN9 data analysis and graphing spreadsheet software allowed equations with slopes to be found that meet both the proportionality and correlation coefficient of their calibration curves. The ORIGN9 spreadsheet utilized in this investigation had fields for absorbance and matching concentrations.

After the measured absorption (the intercept) was entered into the ORIGN9spreadsheet program as requested, the graph, the linear regression equation of the graph inside the slope (b), and the correlation coefficient square of the curve were automatically generated.

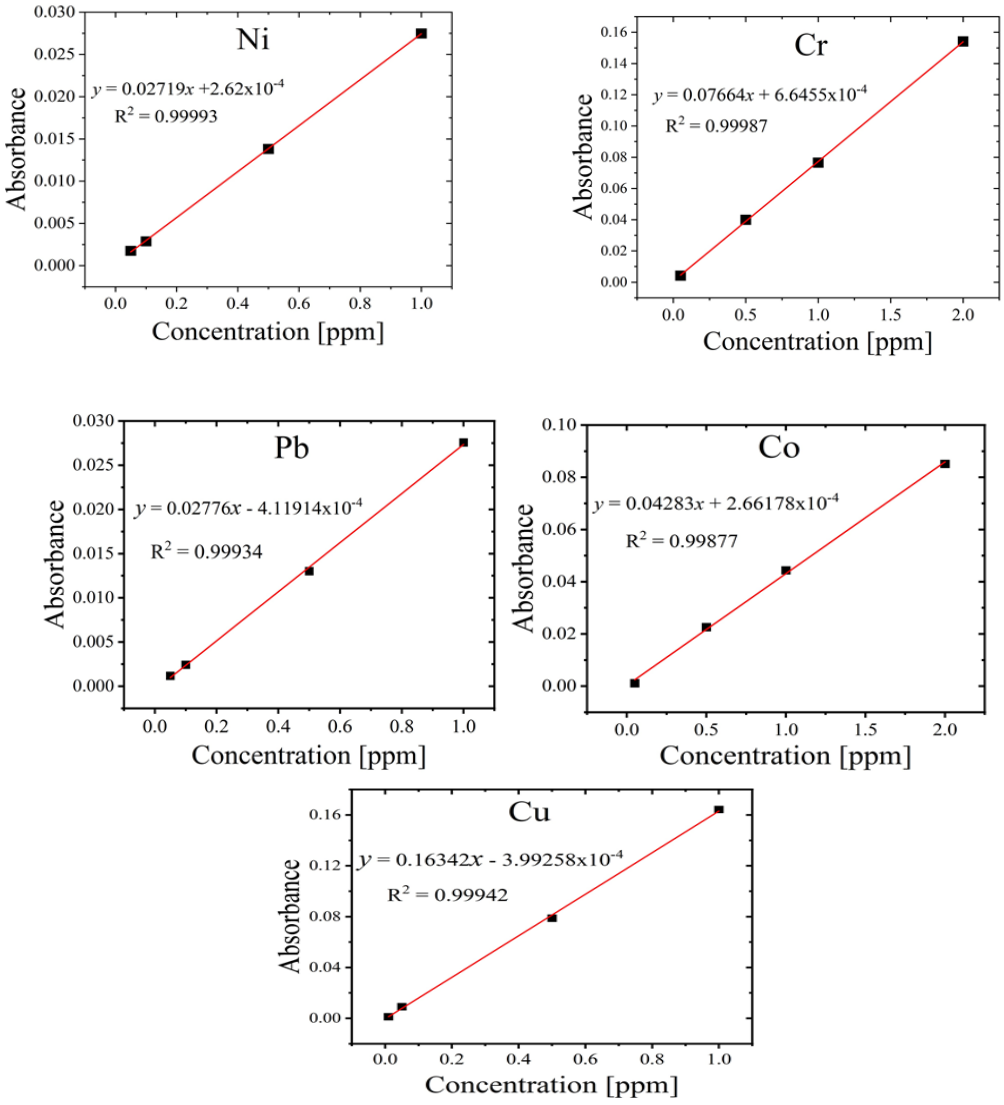

Figure 4.1: Calibration curve of absorbance vs concentration for Ni, Cr, Pb, Co, and Co

The linearity of this plot arises from the beer – lamber's law, which stats that the absorption of light by a substance is proportion to its concentration in solution, given as A= εlC, where A is the absorbance (unite less), ε is molar absorptivity coefficient ($M^{-1}cm^{1}$), is the path length of the light through the cavity (cm), & C is the concentration (m)

4.2 Analysis of malt beverage samples for metal level

The samples were analyzed using flame atomic absorption spectrometer for the determination of lead, chromium, copper, cobalt and nickel at their corresponding wavelength 283.2, 357.9 , 324.7 , 240.7 and 341.5 nm, respectively. The three reading concentration of five heavy metals in the five malt samples determined as the difference of the concentration of diluted malt samples solution for each malt samples, digested three times were shown in table 4.2.

Table 4.2: The three readings of concentration of five heavy metals in each digested samples of the five malt beverage .

Element	Concentration of heavy metals in each digested Sinq malt sample in (mg/L)	Concentration of heavy metals in each digested Sofi malta sample in (mg/L)	Concentration of heavy metals in each digested Bertat malta sample in (mg/L)	Concentration of heavy metals in each digested Nigus malta sample in (mg/L)	Concentration of heavy metals in each digested Malta Guinness sample in (mg/L)
Ni	0.023	0.031	0.016	0.022	0.033
	0.021	0.028	0.015	0.026	0.026
	0.019	0.029	0.013	0.024	0.025
Cr	0.041,	0.031,	0.045,	0.032,	0.051
	0.039,	0.032,	0.043,	0.035,	0.047
	0.040	0.036	0.041	0.031	0.044
Pb	ND	ND	ND	ND	ND
Co	0.051	0.055	0.061	0.071	0.066
	0.049	0.056	0.059	0.073	0.069
	0.043	0.054	0.057	0.076	0.063
Cu	0.681	0.482	0.565	0.626	0.403
	0.687	0.484	0.561	0.651	0.409
	0.683	0.486	0.568	0.657	0.406

The mean level of each five heavy metals (Ni, Cr, Cu, Co and Pb) in the five original malt beverage samples (Sinq, Sofi, Bertat, Nigus and Malta Guinness), those are widely used in our country were expressed in terms of mean ± SD.

Table 4.3: Metals concentration and %RSD in the five malt beverage

Metal	**Concentration of trace metals in Sinq malt**		**Concentration of trace metals in Sofi malt**		**Concentration of trace metals in Bertat malt**		**Concentration of trace metals in Nigus malt**		**Concentration of trace metals in Malta Guinness**	
	Mean ± SD In mg/L	%RSD	Mean ± SD In mg/L	%RSD	Mean ± SD In mg/L	%RSD	Mean ± SD In mg/L	%RSD	Mean ± SD In mg/L	%RSD
Ni	0.021±0.002	9.52 %	0.029±0.015	5.2%	0.015±0.015	10.41 %	0.024±0.02	8.3%	0.028±0.057	15.57 %
Cr	0.04±0.001	2.5%	0.033±0.026	8.02 %	0.043±0.02	4.65 %	0.032±0.02	6.37 %	0.047±0.035	7.42 %
Pb	ND±ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Co	0.048±0.051	8.73 %	0.055±0.01	1.82 %	0.059±0.02	8.47 %	0.073±0.028	3.83 %	0.0666±0.003	4.55 %
Cu	0.683±0.034	0.44 %	0.484±0.02	0.41 %	0.564±0.046	0.815 %	0.644±0.164	2.55 %	0.406±0.03	0.739 %

Mean = $\frac{x}{n}$, Standard deviation σ = $\frac{\sqrt{\sum(x-\chi)^2}}{n}$Where $\sum$ is sum, σ is the standard deviation, x is the value, χ is the mean and n is the total number .

As appeared in Table 4.3, metals (Cr, Ni, Pb, Co, and Cu) were found within the five malt beverage tests. Ni and Cr concentration in Sinq, Sofi, and Malta Guinness were near to the Instrumental discovery limits of FAAS. The concentration level of Co decided within the extend of 0.048 to 0.073 mg/L is the most noteworthy in Nigus consumable beverage (0.073 mg/L), least in Sinq consumable beverage (0.048 mg/L) and medium in Sofi, Bertat, and Malta Guinness consumable beverage (0.055 mg/L,0.059mg/L,0.066mg/L). The concentration of Cr with in the run of 0.032 to 0.047 mg/L is the most elevated in Malta Guinness consumable beverage (0.047 mg/L), the littler within the Nigus malt beverage(0.032 mg/L) and medium in Sinq, Bertat, and Sofi (0.04mg/L,0.043mg/L and 0.033mg/L). The concentration level Cu (0.406

to 0.686 mg/L). The maximum is in Sinq malt (0.686mg/L) and the minimum value is Malta Guinness (0.406mg/L), also the medium is Sofi, Bertat, Nigus (0.484mg/L, 0.564mg/L, 0.644mg/L). The concentration level of Ni (0.015 to 0.029 mg/L), the higher value is in Sofi malt and the minimum is in found in Bertat. The medium is in Sinq, Nigus, Malta Guinness (0.021mg/L, 0.024mg/L, 0.028mg/L) . In this study, Pb is below the detection limit (ND) in the five samples. The essential element cobalt is found above the limit value in Sofi, Bertat, Nigus, and Malta Guinness. In Sinq malt the cobalt heavy malt is below the limit value. Also Ni heavy metal is above the limit value in except Bertat malt beverages.

4.2.1 Comparison of heavy metal levels in the five studied malt beverage samples

As shown in figure below, the concentration of Co decided in Nigus malt test is most elevated (0.073 mg/L) taken then after that Malta Guinness (0.066mg/L), Bertat malt (0.059mg/L), Sofi malt (0.055mg/L) and moderately least sum in Sinq consumable malt test (0.048mg/L). The arranged of levels of Co drink malt beverage tests based on test sorts are Nigus > Malt Guinness >Bertat > Sofi > Sinq. The concentration of Ni decided in Sofi malt test is most elevated (0.029 mg/L) taken after by Malta Guinness (0.028mg/L), Nigus (0.024mg/L), Sinq malt (0.021mg/L) and moderately least sum in Bertat malt test (0.015 mg/L).The arrange of Concentration of Ni in drink beverage tests based on test sorts are Sofi>malta Guinness > Nigus>Sinq> Bertat. The arranged of concentration of Cr within the five tests is Malt Guinness (0.0473mg/L)>Bertat (0.043 mg/L)>Sinq (0.04mg/L)>Sofi (0.033mg/L) > Nigus (0.0326mg/L) .The Cu Concentration arrange within the five test is Sinq (0.686 mg/L) > Nigus (0.644 mg/L) >Bertat (0.564mg/L) >Sofi (0.484 mg/L) > Malta Guinness (0.4406mg/L). The mean concentration of the five follow metals within the five tests of malt beverage can be seen from the bar chart drawn as take after to appear the concentration level of those metals outwardly.

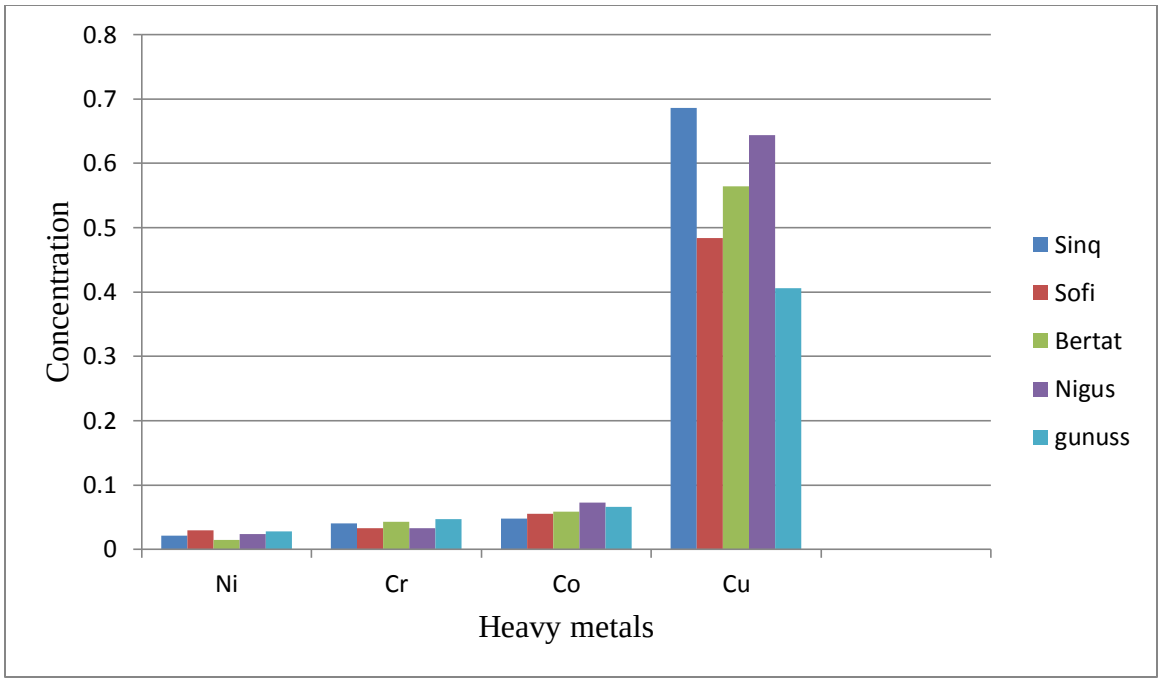

Figure 4.2: Bar chart of mean concentration

As indicated, in table 4.3 and figure 4.1the order of concentration of five heavy metals in Sinq malt samples: Cu (0.683 mg/L) > Co (0.048 mg/L) > Cr (0.04 mg/L) > Ni (0.021 mg/L). The order of concentration of metals in Sofi samples: Cu (0.484 mg/L) > Co (0.055 mg/L) > Cr (0.033 mg/L) > Ni (0.0293 mg/L). In Bertat malt sample the order of concentration of metals given as: Cu (0.564 mg/L) > Co (0.059 mg/L) > Cr (0.043 mg/L) > Ni (0.015 mg/L). The concentration of the Nigus malt: Cu (0.644mg/L) > Co (0.073mg/L) >Cr (0.032mg/L) >Ni (0.024mg/L). Also Malta Guinness: Cu (0.406mg/L) >Co (0.066mg/L) >Cr (0.0473mg/L) >Ni (0.028mg/L). In five malt beverages Pb heavy metal is below the detection limit (ND).

4.2.2 Comparison of the observed Metal levels with recommended International Standards

The comparison of heavy metal content in five malt beverage that was recommended standards that means national and international level . By using the mean value of the five malt beverage heavy metal .

Table 4.4: The mean value of the five malt beverage

Heavy Metal	Total mean value of five malt				
	Sinq	**Sofi**	**Bertat**	**Nigus**	**Guinness**
Ni	0.021	0.029	0.015	0.024	0.028
Cr	0.04	0.033	0.043	0.032	0.047
Pb	ND	ND	ND	ND	ND
Co	0.048	0.055	0.059	0.073	0.066
Cu	0.683	0.48	0.564	0.644	0.44

WHO, Pb = 0.01mg/L, Ni = 0.02mg/L, Co = 0.01mg/L, Cu=2mg/L, Cr =0.05mg/L

Ethiopian standard, Pb= 0.01mg/L, Ni=0.02mg/L, Co= 0.05mg/L, Cu= 1.0mg/L, Cr=0.05 mg/L

In Ni heavy metal except Bertat (0.015mg/L) the four malt such as Sinq, Sofi, Nigus, Malta Guinness (0.021mg/L, 0.029mg/L, 0.024mg/L, 0.028mg/L) is above the maximum permission value of WHO and above permission value of Ethiopian standards. The Cr, Sinq, Sofi, Bertat, Nigus, Malt Guinness (0.04mg/L, 0.033mg/L, 0.043mg/L, 0.032mg/L, 0.047mg/L) below the permission limit of WHO respectively . In Cu metal in five malt, Sinq, Sofi, Bertat, Nigus, Malt Guinness (0.683mg/L, 0.48mg/L, 0.564mg/L, 0.644mg/L, 0.44mg/L), all are below the permission value of WHO and Ethiopian standards. Also in Co, in all malt beverages it's above the permit value international and nationals, but the Pb heavy metal is below the detection limit.

4.3 Validation of experiment results

4.3.1 Accuracy

The whole expository strategy was performed for all of the chosen metals in triplicate spiked tests of malt and three perusing of concentration of each metal was decided. The concentration of all chosen metals in triplicate spiked malt tests displayed in Table 4.5 underneath.

Table 4.5: Concentration of selected heavy metals in triplicate spiked of Sinq malt samples determined from FAAS in (mg/L)

Heavy metal	**Ni**	**Cr**	**Pb**	**Co**	**Cu**
Concentration	0.120	0.139	0.118	0.145	0.781
of spiked	0.119	0.138	0.117	0.146	0.779
sample (mg/L)	0.117	0.140	0.115	0.144	0.782
Amount of added in(mg/L)	0.10	0.10	0.10	0.10	0.10

The accuracy and validation of procedure was evaluated by analyzing the mean concentration of the five heavy metal in triplication spiked malt sample and the mean concentration in selected non-spiked malt sample. The result of recovery analysis determination for five metals in the spiked malt sample we give in table 4.6.

Table 4.6; Recovery test for the optimized procedure for Sinq malt sample

Metal	**Concentration in (mg/L)**			**Recovery %**
	Un spiked	**Amount of added**	**Spiked sample**	
Ni	0.021	0.10	0.119	98
Cr	0.041	0.10	0.139	98
Pb	0.019	0.10	0.117	98
Co	0.047	0.10	0.145	98
Cu	0.683	0.10	0.780	97

The results of percentage recoveries for the studied metals were all ranged between 97 - 98 %. Generally good recoveries were obtained for all metals. The results of the recovery tests for samples were within the acceptable range for most metals 85-103% [62].

By comparing the measured concentration of the spiked analyte with its known concentration, the accuracy of the analytical method can be evaluated. The measured concentration closely matches the spiked concentration, the method is considered accurate. So the known concentration of the Ni, Cr, Pb, Co, and Cu (0.10, 0.10, 0.10, 0.10, and 0.10) and the measured spiked concentration is also for each heavy metal (0.119, 0.139, 0.117, 0.145, and 0.780) respectively. We conclude that our experimental result was considered to be an accurate because the known concentration closed matches to the spiked concentration.

4.3.2 Precession

The precision of the results determined in this work also described by the standard deviation. The precision of the measurements can be determined by examining the standard deviation (SD) value associated with each mean concentration. Here are the precision values for each metal:

1. Nickel(Ni):

Precession: ±0.002mg/L, ±0.0015mg/L, ±0.0015mg/L,±0.002mg/L, ±0.0057mg/L

2. Chromium(Cr):

Precision: ±0.001mg/L, ±0.0026mg/L, ±0.002mg/L, ±0.002mg/L, ±0.035mg/L

3. Lead(Pb):

Precession: Not Determined (ND)

4. Cobalt(Co):

Precession: ±0.0051mg/L, ±0.001mg/L, ±0.002mg/L, ±0.0028mg/L, ±0.003mg/L

5. Copper(Cu):

Precession: ±0.0034mg/L, ±0.002mg/L, ±0.0046mg/L, ±0.0164mg/L, ±0.003mg/L

Precision is indicated by the standard deviation values, showing the degree of variability or spread of the measured concentrations around the mean value. This shows that the variation might be occur between the repeated measurements should be very small as the standard deviation of all elements found in the range of min SD to max SD for Ni, Cr, Pb, Co, and Cu

(0.0015mg/L to 0.0057mg/L, 0.001mg/L to 0.035mg/L, ND, 0.001mg/L to 0.0051mg/L, and 0.0026mg/L to 0.0046mg/L) respectively. For all metal mean concentrations except nickel, the computed relative standard deviations (% RSD) are less than 10%. In Bertat malt and Malta Guinness, the relative standard deviations (% RSD) of Ni are 10.41 and 15.57, respectively, although they are fewer than 10 in Sinq, Sofi, and Nigus malt. Consequently, good instrumental precisions are shown in the relative standard deviations (% RSD) result.

4.3.3 Linearity

Linear regression equation of known concentration of standard solution to the Ni, Cr, Pb, Co, and Cu (y=0.0272x+0.0003, y=0.0766x+0.0007, y=0.0278x-0.0004, y=0.0428x+0.0003, and y=0.1634-0.0004) respectively. The level of heavy metal that are Ni, Cr, Pb, Co, and Cu value of R^2 (0.9999, 0.9998, 0.9993, 0.9988, and 0.9994) they are linear because the value of R^2 is close to 1. The concentration of the heavy metal is proportional to the absorbance that means they have strong linear relationships. A high R^2 value close to 1 indicates that the data points very close to the fitted regression line, demonstrating excellent linearity and the reliability of analytical method used. This value is highly accurate and precise in quantifying the concentration of these metals. The measure of the strength of the degree of correlation between the instrument response (absorbance) and the known concentration were good for all metal as shown in table 4.1 for each metal. This shows that a good linearity between absorbance and concentration as the correlation coefficients of all elements found in the range of ($0.9999 \geq 0.9988$).

4.3.4. Method of detection limit (MOD)

The method of detection limit in five heavy metals are calculated below in Table 4.7

Table 4.7: The method detection limit of the studied heavy metal

Metal	Sinq	Sofi	Bertat	Nigus	Malta Guinness
Ni	0.018	0.0135	0.0135	0.018	0.0387
Cr	0.009	0.0234	0.018	0.018	0.0315
Pb	ND	ND	ND	ND	ND
Co	0.0378	0.009	0.018	0.0225	0.027
Cu	0.030	0.018	0.041	0.21	0.027

The standard deviation (of the results of triplicate measurements of a given solutions were calculated for all studied metals. The MDL was calculated as (MDL= σ × n) where n = 9, and compared with Instrumental detection limit [63].

The method detection limit of the studied heavy metal suggested that the method of detection limit of each element is above the detection limit except in Cu metal. Therefore sensitive analytical method is good for all studied heavy metals.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

In this study, the concentration of five heavy metals (Cr, Co, Pb, Cu and Ni) in the five different malt beverage samples (Sinq, Sofi, Bertat, Nigus and Malta Guinness) collected from the supermarket and grocery in Hawassa, Ethiopia were analyzed using flame atomic absorption spectroscope. The five malt beverage samples had concentrations of Co, Cr, Cu, and Ni ranging from 0.048 to 0.073 mg/l, 0.032 to 0.047 mg/L, 0.406 to 0.683 mg/L, and 0.015 to 0.028 mg/L, respectively. Variable species of malt drinks had somewhat variable element concentrations, which could be explained by the diverse ways in which different heavy metals are absorbed by malt. In Sinq malt sample the concentration of heavy metals are: Cu (0.683 mg/L) > Co (0.048 mg/L) > Cr (0.04 mg/L) > Ni (0.021 mg/L). Sofi malt samples had the following concentrations of heavy metals: Cu (0.484 mg/L) > Co (0.055 mg/L) > Cr (0.033 mg/L) > Ni (0.0293 mg/L). In Bertat malt sample: Cu (0.564 mg/L) > Co (0.059 mg/L) > Cr (0.043 mg/L) > Ni (0.015 mg/L). The concentration of heavy metals in Nigus malt are also in the order of: Cu (0.644mg/L) >Co (0.073mg/L) >Cr (0.032mg/L) >Ni (0.024mg/L). Similarly, in Malta Guinness sample the level of heavy metals are in the order of: Cu (0.406mg/L) >Co (0.066mg/L) >Cr (0.047mg/L) >Ni (0.028mg/L). On the other hand, the level of Lead (Pb) in all of the malt samples is below the detection limit (ND). The level of Cobalt (Co) concentrations are in the range between 0.048 mg/L to 0.073 mg/L, the remaining five malt drinks contain slightly higher cobalt concentrations as compared to the maximum limit set by WHO (0.01 mg/L). The Ni level obtained in the beverages range between 0.015 mg/L and 0.029 mg/L, which is above the maximum limit set by WHO (0.02 mg/L) except the Bertat that has concentration range (0.015mg/L). The concentration of Cu is in the range from 0.406 mg/L to 0.683 mg/L, which is much smaller than the maximum allowed value set by WHO (2.0 mg/L). The level of Cr in the samples range between 0.032mg/L and 0.047mg/L which is again below the maximum allowed limit set by WHO (0.05 mg/L). The experimental results are validated in terms of accuracy, precession, linearity and limit of detection. As a result, the analytical method's sensitivity was appropriate for every study element. The percentage recoveries obtained for the metals under investigation

varied from 97% to 98%. By comparing the measured concentration of the spiked analyte with its known concentration, the accuracy of the analytical method can be evaluated. The precision of the results were evaluated by the relative standard deviation of the results of triplicate digests and percentage relative standard deviation of the results of five samples (N = 5). The calculated values of relative standard deviations (% RSD) are less than 10% for all of the mean concentrations of metals except Ni. Relative standard deviations (% RSD) of Ni, however, are less than 10 in Sinq, Sofi, and Nigus malt, and10.41 in Bertat and 15.57 in Malta Guinness. The five heavy metals have good linearity between absorbance and concentration as the correlation coefficients of all elements found in the range of ($0.9999 \geq 0.9988$). The method detection limit of the studied heavy metal suggested that the method of detection limit of each element is above the detection limit except Cu metal. Therefore sensitive analytical method is good for all studied heavy metals.

5.2 Recommendation

In the work of this study findings and the conclusion drawn above, the following recommendations are forwarded.

This research adds a great deal to the body of knowledge regarding the safety of malt drinks and offers useful data that can be applied to safeguard the general public's health. AAS was utilized in this study to examine the concentrations of heavy metals in Ethiopian malt drinks. They discovered that all of the Co and Ni concentrations in five malt drinks were small difference to limits established by the WHO and the Ethiopian Food and Drug Administration. As a result, I recommended that the manufacturers of malt beverages Company pay attention to continuous monitoring the sources of these metals in malt beverages.

This thesis work might be repeated with GFAAS, and ICP-OES to compare the metal concentration of the five malt samples. There is a gap of knowledge on the heavy metal content of malt beverage used in our country. So that determining the concentration of heavy metals in different malt beverage used in our country should be encouraged.

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APPENDICS

Appendix 1

Figure1: Samples taken from different area

Appendix 2

Figure 2:- Buck Scientific, Model 210VGP AAS, USA

Figure 3: Sample on hot plate

Figure4: Sample taken to the nebulizer (to atomize)

Figure 5: A screen display

Figure 6: During experimental laboratory room for measuring each sample.