

DETERMINATION OF HEAVY METALS CONCENTRATION IN
DIFFERENT BRANDS OF BOTTLE WATER AVAILABLE IN ETHIOPIAN
MARKETS BY USING FLAME ATOMIC ABSORPTION SPECTROSCOPY



M Sc. IN LASER PHYSICS (LASER SPECTROSCOPY)

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ABSTRACT

Flame atomic absorption spectrometry (FAAS) is a crucial analytical technique widely used in environmental studies to detect and quantify heavy metals. In this research we used FAAS to determine the concentrations of Ni, Cu, Cr, Co, and Pb in 18 different brands of bottled water collected from Ethiopian's markets. Samples were stabilized with 2% nitric acid and analyzed using flame atomic absorption spectroscopy (FAAS) after precise calibration with standard solutions. The concentrations of Ni, Cu, Cr, Co, and Pb ranged from 0.021 - 0.068 mg/L, 0.021 - 0.084 mg/L, 0.044 - 0.049 mg/L, 0.00 mg/L, and 0.005 - 0.0093 mg/L respectively. The method validation demonstrated excellent linearity, with correlation coefficients (R^2) of 0.9994, 0.9995, 0.9998, 0.9990, and 0.9999 for Ni, Cu, Cr, Co and Pb, respectively. Precision (%RSD) was within acceptable limits ranging from 0.21% to 8.5% for Ni, 0.0% to 8.8% for Cu, 0.2% to 9% for Cr, and 0.0% to 13.04% for Pb. These values are acceptable as compared to the standard acceptable value of $R^2 > 0.999$ for linearity and $\%RSD \leq 16\%$ for precision based on ICH value. Recoveries (accuracy) ranged from (92.6 - 101.7) % which is within the acceptable range of recovery test of 90% - 110% based on ICH values. The concentration of heavy metals found in all bottled water samples are below the recommended World Health Organization's (WHO's) and Ethiopian's quality guidelines for drinking water for heavy metals so that there is no safety compliance with respect to this heavy metals.

Keywords: FAAS, Light-matter interaction, Beer-Lambert law, Bottled Spring drinking water, heavy metals.

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LIST OF ABBREVIATIONS

AAS.....	Atomic absorption spectroscopy
UV-VIS.....	Ultra violet – visible
FT- IR.....	Fourier transform- infrared
ICP- MS.....	Inductively coupled plasma mass spectroscopy
ICP.....	Inductively coupled plasma
MS.....	Mass spectroscopy
ppb.....	Parts per billion
ppm.....	Parts per million
NMR.....	Nuclear magnetic resonance
PET.....	Polyethylene terephthalate
WHO.....	World health organization
LIBS.....	Laser induced breakdown spectroscopy

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Spectroscopy is the science of studying the properties of atoms or molecules through their interaction with electromagnetic radiation at specific wave lengths or frequencies (Dadi & Yasir, 2021). During these interactions the light energy is either absorbed, emitted, or scattered, which is represented by spectra. The absorption or emission spectra obtained from the spectrometric techniques provide valuable information on the type of chemical compounds, concentrations of elements, type of functional groups, and kinds of bonds present in a sample (Bernath, 1995).

Based on the frequency or wavelength range of an electromagnetic radiation used and the information need to extract from our sample, commonly used spectroscopic techniques include Atomic Absorption Spectroscopy (AAS), Atomic Emission Spectroscopy (AES), UV-VIS Spectroscopy, FT-IR Spectroscopy, Raman Spectroscopy, and Icp/ Icp- Ms Spectroscopy. These techniques function by either absorption, emission or scattering of electromagnetic radiation and can be categorized accordingly: absorption spectroscopy (AAS, FT-IR, NMR), emission spectroscopy (spectrofluorimetry), and scattering spectroscopy (Raman spectroscopy) (Hollas, 2005; Mohammed, 2021).

In general, as compared to the conventional analytical techniques, spectroscopic techniques offer advantages such as rapid analysis result in a matter of seconds, high sensitivity due to advanced technology, and real time monitoring in manufacturing process (Mohammed, 2021).

The analytical atomic absorption spectrometer was developed by Alan Walsh in 1953 for the analyzing of very small number of metallic elements in a sample by implementing radiation absorption by atomic vapors. Since then, AAS has become a powerful analytical instrument in most laboratories (Mohammed, 2021).

AAS, which operates in the UV- VIS range of an electromagnetic radiation, is most widely used analytical technique by most researchers for detecting and measuring trace metallic elements in environmental samples due to its high sensitivity (down to parts per billion), low cost per analysis, ease of operation, straightforward sample preparation, and minimal inter-elemental interference (Bernath, 1995; Mohammed, 2021). The technique works by transitioning atoms from the ground state to an excited state through the absorption of radiant energy that matches the energy difference between these states. This principle is fundamental in analyzing heavy metals in various environmental samples (Bernath, 1995; Mohammed, 2021).

AAS is utilized to determine the concentration of metal atoms in different environmental samples. Metals make up 75% of Earthstar's chemical elements, making them a significant concern for human health. While metal content is sometimes desirable, it can also be contaminating and hazardous (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Therefore, measuring metal content is critical for various applications.

Some of the application areas of AAS include pharmaceuticals (Rao et al., 2010), mining (Fouad et al.,2015), food and beverage industries (Alkis et al.,2014), and forensic investigations (Maurya et al.,2018). AAS has also been extensively used to analyze heavy

metal concentrations in drinking water for quality control, ensuring it meets the required safety standards for drinking water.

Currently, environmental pollution by heavy metal is current global issue that needs to be addressed with proper analytical based research for the sake of the societal health (Luqueño et al.,2013).

Heavy metals, defined as metals with atomic density greater than 4 g/ cm^3 or five times that of water, includes elements such as cadmium, zinc, mercury, arsenic, silver, chromium, copper, iron and platinum group elements (Das et al.,2011). While some heavy metals are necessary in small amount for health, they became toxic above certain concentration limits that are set by WHO, including copper, zinc, manganese, iron, nickel, cobalt and chromium. Others, like arsenic, lead, mercury and cadmium, are highly toxic even at low concentrations (Durube et al.,2007). Heavy metals enter the environment through both human activities and natural process.

Human activities contributing to heavy metal pollution include emissions from mining, smelting, battery manufacturing, tanneries, paint production, pesticides, fertilizers, and printing or photographic industries. Natural processes include weathering, volcanic activities, and the chemical composition of aquifers, which can enrich water sources with heavy metals (Tchounwou et al., 2012; Olowoyo et al., 2022). Consequently, soils become major deposition area for heavy metals, which eventually lead to aquatic systems. Packaging materials such as polyethylene terephthalate (PET) and recycled PET (R-PET) bottles contribute to contamination through the leaching of heavy metals like antimony from antimony trioxide, a catalyst used in PET production (Shotyk & Krachler, 2007). As the

result, heavy metals pose a significant global health risk as they are non- biodegradable and persist in the environment for a long period of time (Das et al., 2011; Durube et al., 2007).

One method that enters to our body is through drinking water. Excessive levels of heavy metals in drinking water can have various adverse effects on human health. Including internal organs damage, nervous system impairment, and cancer (Durube et al., 2007; Mekonnen & Surur, 2015). Due to these effects, it is critical to address heavy metal contamination in drinking water in Ethiopia by using appropriate analytical techniques.

Bottled waters come from different sources like springs, and underground water sources (Toma, 2011). One of the drinking water available in Ethiopian market is bottled water with a source that varies from natural spring water, purified underground water, to mineral water which is then treated and processed before being packed for the market.

The number and type of bottled water production and consumption in Ethiopia is increasing from time to time, since it is assumed as one of the purest forms of drinking water with no contaminants. However, studies conducted round the world on bottled water by using spectroscopic technique have found heavy metals in it. These studies have been conducted in Canada (Pip, 2000), Uganda (Bamuwanye et al., 2011), Nepal (Gautam, 2020), Nigeria (Maxwell et al., 2018), Germany (Shotyk & Krachler, 2007), California,UAS (Sullivan, 2011), Iraqi (Toma, 2011), and South Africa (Olowoyo et al., 2022). Some of these studies detected heavy metals in some bottled water above the permissible international limits. In Legos, Nigeria Lead was detected with maximum value of 0.348mg/l; In Pretoria, South Africa Lead and Chromium were detected with maximum value of 0.123mg/l and 0.107mg/l respectively; In Kampala, Uganda Lead and Chromium were detected with maximum value of

0.241mg/l and 0.107mg/l respectively; and In Delhi, India Lead was detected in the maximum value of 0.058mg/l. The studies also have shown that there is variation in the concentration of heavy metals in bottled waters depending on their origin, storage conditions, and processing technology (Das et al., 2011). Hence regular monitoring of the qualities of bottled water is necessary to ensure that heavy metal concentrations remain within a safe limit over time.

Therefore, the present study aims to analyze heavy metal content of the bottled waters available in Ethiopian market by using Flame Atomic Absorption Spectroscopy (FAAS). The study involves sample preparation, instrument calibration, measurement, and validation of methods to ensure reliability.

1.2 Statements of the Problem

In Ethiopia, the number of water bottling companies producing different brands of bottled water is growing. These companies are expected to provide comprehensive information about the quality of their products, including heavy metal content, to ensure safety for consumers.

However, the researcher observed that different companies in Ethiopia are producing different brands of bottled water with a stamped trade mark that describes the different parameters of the bottled water and concentration of major elements but information concerning the heavy metal contents of the water is ignored.

Few studies have been conducted to address the chemical quality of bottled water in Ethiopia, such as those examining the level of common ions in bottled mineral water consumed in Addis Ababa (Yilkal et al., 2019), and levels of common ions in bottled mineral waters consumed in Addis Ababa (Seda et al., 2013), the assessment of chemical quality of major

brands of bottled natural spring water marketed in Gonder (Mekonnen et al., 2015), and chemical analysis of major brands of bottled mineral water available in Ethiopian market (Mitiku, & Endeshaw, 2022). However, these studies have not focused on the heavy metal content of bottled natural spring water consumed in Ethiopian market. Moreover, they were conducted by departments other than physics, and focused on specific geographic areas. This highlights a gap in literature, particularly from a physics department, on heavy metal detection in bottled water using spectroscopic techniques.

As we can see, if the heavy metal issue is left unchecked for a long period of time, then it will greatly affect the health of the society in different ways. (Luqueño et al., 2013). Intern this also greatly affects the country's economy. After all no one has the time and resource to reverse the damage it results on the society's health unless fast remedial action is taken now.

Therefore, this research aims to determine concentrations of some selected heavy metals in different brands of bottled water available in Ethiopian market by using atomic absorption spectrometry, applying the correct spectroscopic techniques and principles.

1.3 Objectives

1.3.1 General Objective

The main objective of this thesis is to apply flame atomic absorption spectroscopy to determine the concentration of heavy metals in different brands of bottled water available in the Ethiopian market.

1.3.2 Specific Objectives

The specific objectives of the study are:

- ✓ To determine the concentration of each heavy metal in the bottled drinking water samples collected from different geographical locations
- ✓ To compare the results with international and national drinking water quality standards and related studies.
- ✓ To validate the analytical method and results of the analysis

1.3.3 Research Questions

This research aims to answer the following questions:

- How is FAAS technique is used for quantitative analysis of heavy metals in drinking water?
- How is the analytical process validated?
- Are the bottled drinking waters available in Ethiopian markets safe, interims of the detected heavy metals content?

1.4 Significance of the Study

Bottled drinking water has come as an essential commodity and consumed by a large number of people especially in areas where clean drinking water access is limited. Since it is assumed as one of the purest and safest water sources for drinking, its quality must be assured, particularly regarding heavy metals, to protect public health.

Besides to this, bottled drinking water can serve as source of foreign currency income for the country through export trade to different countries all over the world if its quality is properly assured and certified. Therefore, this study in general serves as an important quality control indicator by producing reliable information on the concentration of heavy metal on each sample of bottled water and make companies produce and deliver their product based on

WHO and other countries quality control base line and to create competition among them. And also, the researcher aims to disseminate the result of this study for quality regulators, bottling water companies, policy makers and implementers to indicate how serious the issue is.

Finally, this study would bridge the literature gap in physics department specifically on laser spectroscopy application, instrumentation, working principles and the physics behind the atomic absorption spectroscopic techniques, since, up to my knowledge, there is limited resource on it. And also serve as bench mark for other researchers to extend the study by using a powerful and most sensitive analytical technique on determination of other heavy metals on bottled water for quality controlling activity.

1.5 Scope of the Study

This study was conducted by collecting eighteen samples of bottled water consumed in Ethiopian market which are produced by companies located in different geographic locations. From Northern Ethiopia- East Amhara region around Debrebrhan, and West Amhara region round Bahr Dar; from Southern Ethiopia- Sidama region round Hawassa, south oromiya around Arisi zone; from West Ethiopia- west oromiya around Jimma zone; from Central (Meakelawi) Ethiopia region around Welkite zone, Silte zone, and around Addis Abeba. The collection of the samples was done from September to July 2016 E.C

Then the analysis was done by using the available state of art- flame atomic absorption spectroscopic technique to determine the concentration of only selected five heavy metals.

1.6 Limitation of the Study

Thus, due to the budget constraint the study was limited to determine the concentration level of only selected heavy metals (Ni, Cr, Cu, Co and Pb), which may not capture the full spectrum of potential contaminants, in the collected bottled water samples from different markets in Ethiopia. Additionally, the study was limited to 18 bottled water brands, which, while representative, may not encompass all the bottled water products available in the Ethiopian market. And finally, due to the model of the FAAS instrument the absorbance spectral lines were not available.

CHAPTER TWO

THEORETICAL BACKGROUND

2.1 Spectroscopy

Spectroscopy is the science of studying the properties of matter, such as atoms and molecules, through their interaction with light (Dadi et al., 2021). Light matter interaction results in two types of spectra: atomic and molecular. Analyzing spectral graphs obtained through various spectroscopic techniques and types of electromagnetic radiation provides qualitative and quantitative information about the absorbing or emitting entities contained in a sample (Bernath, 1995; Hollas, 2005).

Some of the commonly used spectroscopic techniques include atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), inductively coupled plasma spectroscopy (ICP), Fourier transform infrared ray spectroscopy (FT- IR), Raman spectroscopy, and laser induced break down spectroscopy (LIBS). These techniques are grouped in to absorption, emission, and scattering spectroscopy based on the interaction methods (Hollas, 2005; Mohammed, 2021).

Absorption spectroscopy measures the absorbed electromagnetic radiation by matter as a function of wavelength, as seen AAS and FTIR spectroscopy. Emission spectroscopy such as AES and LIBS, studies the emission of electromagnetic radiation by excited atoms or molecules. Scattering spectroscopy, like Raman spectroscopy, utilizes the inelastic scattering of monochromatic light by molecules to obtain vibrational, rotational, and other molecular

information. These methods are used to study electronic transitions in atoms; vibrational, rotational, and electronic transitions within molecules (Bernath, 1995; Hollas, 2005).

Spectrum is a graphical representation of how the sample interacts with light. It can be plotted as transmittance or absorbance versus wave length or wave number (Bernath, 1995). By interpreting spectra, we can obtain qualitative and quantitative information about matter. Spectroscopic techniques offer significant advantages over the conventional analytical methods, such as providing rapid analytical results, requiring only small sample amount, offering better detection limits due to advanced technology, enabling real-time monitoring of the manufacturing process without the need of sample preparation, and providing both quantitative and qualitative information (Tchounwou et al., 2012).

As the result, spectroscopy finds applications in various fields, including environmental monitoring, quality assurance in food and beverages, pharmaceutical analysis, forensic science, astronomy, minerology, and material characterization (Dadi et al., 2021; Bernath, 1995). This chapter discusses the theory of light matter interaction, Beer-Lamberts law, the basics of atomic absorption spectroscopy, such as working principle and instrumentation, Boltzmann's distribution, and the application of atomic absorption spectroscopic techniques for the determination of heavy metals in bottled water. In addition, the growing concerns of environmental contaminations particularly by heavy metals in drinking water and possible sources of these contaminations is discussed.

2.2 The Physics of Light Matter Interaction

When electromagnetic radiation interacts with matter (atoms or molecules), absorption, emission, or scattering phenomena occur. Matters interact with the UV/VIS range of an electromagnetic radiation, leading to absorption transition from the ground state to the excited state. This section presents the dual nature of EM radiation, the electromagnetic spectrum, and the energy levels of mater.

2.2.1 Electromagnetic Radiation (EMR)

Electromagnetic radiation (EMR) is radiant energy produced by the accelerated motion of an electric charge, such as an electron, in an electric field. EMR propagates outward in all directions through space, either with or without a material medium (Dadi et al., 2021; Hollas, 2005).

EMR can be described in two different ways: as particles (photons) and as wave. The particle nature of light suggests that it acts as particles with definite energy packets called quanta or photons. The energy of n photons is expressed by plank's equation as.

$$E = n h\nu, \quad (2.1)$$

where h is plank constant ($h= 6.63 \times 10^{-34}$ J.s), ν is the frequency of radiation, and n is the photons, which determines the intensity of radiation. The wave nature is described by the transverse oscillation of electric and magmatic fields, both oscillating with same frequency, perpendicular to each other and to the direction of propagation.

Light can be polarized, meaning the electric and magnetic fields oscillate in one particular direction. Unpolarized light has electric and magnetic fields oscillating in all directions perpendicular to the direction of propagation. The frequency (ν) and wavelength (λ) of a light are related to by:

$$c = \lambda \nu, \quad (2.2)$$

where c is the velocity of propagation of the wave, for electromagnetic radiation in vacuum, $c = 3 \times 10^8$ m/s. Frequency and wavelength are used to characterize the wave nature of an EMR. EMR is often illustrated by considering plane-polarized (linearly polarized) radiation. Figure 2.1 illustrates one photon of such radiation travelling along the $+x$ direction. The electric component of the radiation forms an oscillating electric field of strength \mathbf{E} , and the magnetic component forms an oscillating magnetic field of strength \mathbf{H} . If the directions of the vectors \mathbf{E} and \mathbf{H} are y and z respectively, then

$$\mathbf{E} = E_0 \sin(\omega t \pm kx), \quad (2.3)$$

$$\mathbf{H} = H_0 \sin(\omega t \pm kx), \quad (2.4)$$

where E_0 and H_0 are the amplitudes, and ω is angular frequency. The fields oscillate sinusoidally with a frequency of $2\pi\nu$ and because k is the same for each component, they are in-phase. The plane of polarization is conventionally taken to be the plane containing the direction of \mathbf{E} and that of propagation; as shown in Figure 2.1, this is the x - y plane. The amplitudes of electric field and magnetic fields are related by $E_0/B_0 = c$ which explains why magnetic interactions are much weaker than the electric interactions. Therefore, the

interaction of electromagnetic radiation with matter is primarily through the electric component.

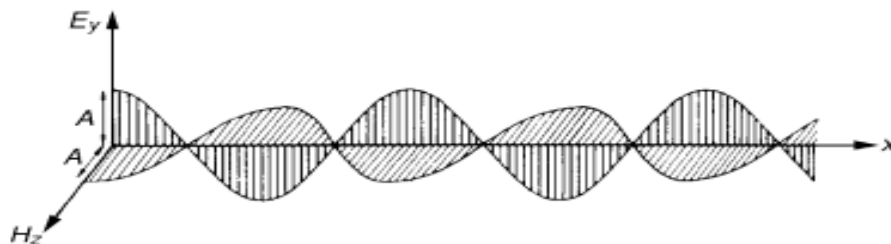


Figure 2.1: Polarized Electromagnetic wave (Hollas, M. J., 2005).

2.2.2 Electromagnetic Spectrum

Different regions of EM waves have unique names and functions in spectroscopy. These regions include:

- Radio frequency region (3MHz- 3GHz): Cause the nuclear magnetic resonance (NMR) by flipping nuclear spins.
- Microwave region (3GHz-3000GHz): Induces rotational transitions in molecules and cause electro spin resonance in far microwave region.
- Infrared region (100 cm^{-1} - $13,000\text{ cm}^{-1}$): Induce vibrational motion in matter.
- Visible and ultraviolet (uv) region ($10,000\text{ \AA}$ - 100 \AA): cause molecular band electronic transitions.
- X-ray region (100 \AA - 0.1 \AA): cause core electronic transitions.
- Gama-ray region (less than 0.1 \AA): Related to nuclear process (Bernath, 1995).

The different regions of the spectrum do not possess sharp boundaries; they overlap and are approximate as shown in the figure bellow.

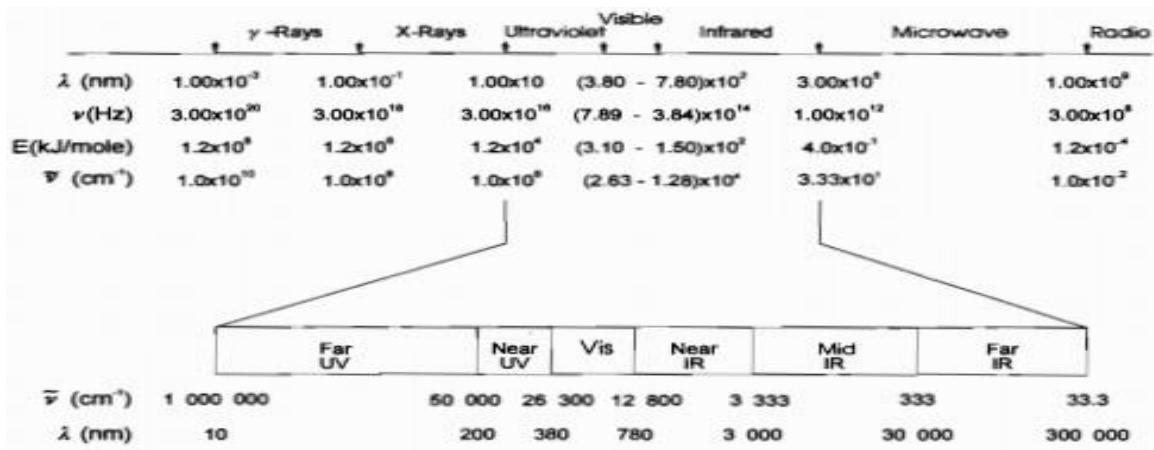


Figure 2.2: Electromagnetic spectrum (Milionni, & Eberly, 2010).

The visible region is further divided into seven colors, ranging from violet (4000Å) to red (7800Å). The UV range is also divided as near ultraviolet region (4000Å - 200Å) and vacuum UV, that is used for spectroscopic techniques only in evacuated instruments (Milionni, et al., 2010; Hollas, 2005).

2.2.3 Energy Level of Matter and Selection Rule

The energy levels of matter (atoms or molecules) vary from the simple two-level systems for lighter atoms to complex many-level systems for heavier atoms. Each level is associated with a fixed energy gap. The lowest, most stable configuration of a matter is called ground state.

When appropriate radiant energy is supplied, matter absorbs the energy and undergoes excitation from the ground state to a less stable excited state. The life time at the excited state is very short, so the system returns to a stable state spontaneously by emitting radiant energy equivalent to the absorbed amount. Heavier atoms have complex electronic structures with many electronic transitions, each associated with the absorption of specific energy related to each energy gap (Milionni, et al., 2010).

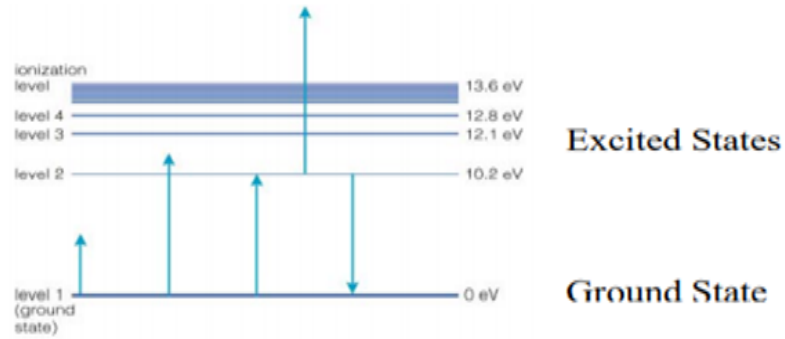


Figure 2.3: Energy level of matter (Allowed transition, 2017).

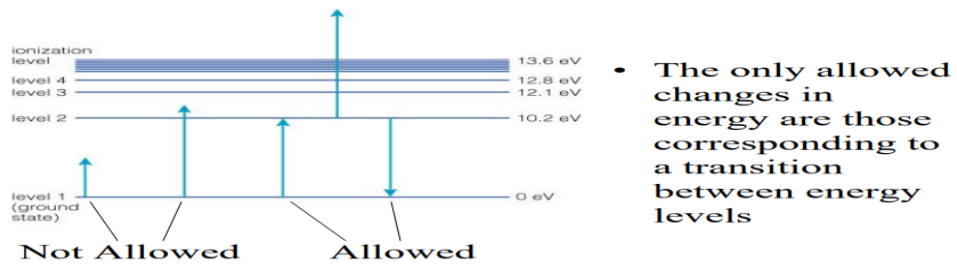


Figure 2.4: Energy level transition in matter. (Allowed transition, 2017)

The selection rule describes the allowed and forbidden transitions between quantized energy states in matter. When the energy of absorbed radiation equals the energy gap of a given state, maximum absorption occurs, resulting in an allowed transition. This allowed absorption phenomena are represented graphically by a line spectrum between two accessible energy states as shown in Figure 2.4

2.2.4 Boltzmann's Distribution

Boltzmann distribution describes the relative population of different energy levels in matter. It relates the distribution to the thermal temperature (Lagalante, 1999). By considering a two-level atomic system, if E_1 and E_2 are the energy of ground and excited states respectively, with

corresponding n_1 and n_2 numbers of atoms and electrons, then the ratio of the number of atoms in the excited states to the ground state is given by Boltzmann equation as:

$$\frac{n_1}{n_2} = \frac{g_1}{g_2} e^{-\frac{\Delta E}{kT}} \quad (2.5)$$

Where T is temperature, g_1 and g_2 statistical weights of individual states, K Boltzmann constant, $\Delta E = E_2 - E_1 = h\nu = hc/\lambda$ is the energy difference between states, with c , ν , λ representing the speed of light, frequency, and wavelength of the absorbed radiation respectively.

For example, gases used for the burning system in FAAS is air – acetylene or nitrous oxide-acetylene with operating temperature 2400 °C and 2800 °C respectively. This flame temperature in FAAS is adjusted only to atomize the sample and produce ground state atoms in gaseous form but not to excite it, so the flame temperature is relatively colder. Then from Max-Well Boltzmann equation at typical flame temperature ($E \gg kT$) most of the atoms are found in the ground state than the excited state ($n_1 \gg n_2$). Therefore, the transition from the ground state to the excited state (absorption) depends on energy of incident radiation from the light source.

In general, Boltzmann distribution explains the population distribution of atoms in different energy states in a flame that affect absorption in FAAS (Lagalante, 1999; Mohammed, 2021).

2.2.5 Beer-Lambert's Law

The Beer-Lamberts law establishes a relation between absorbance and concentration, as well as between absorbance and the path length of the absorbing medium. In FAAS spectroscopy, when a parallel beam of radiation with an initial intensity I_0 is directed through an atomic gas

or vapor in its ground state over a path length L , the intensity of the light decreases as it passes through the sample. The relationship between the initial intensity I_0 and the transmitted intensity I is given by:

$$I = I_0 e^{-klc}, \quad (2.6)$$

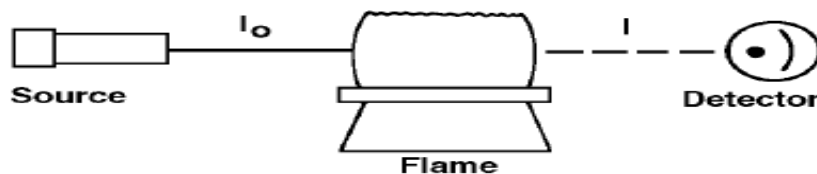


Figure 2.5 : Comparison of incident radiation with transmitted radiation (Lagalante, 1999).

The transmitted light intensity is measured by the detector and compared with incident radiation to determine the absorbed intensity, which corresponds to the concentration of atoms in a sample.

The transmittance T , which is the ratio of the transmitted light intensity I to the incident light intensity I_0 , is given by:

$$T = I / I_0, \quad (2.7)$$

In practical applications, absorbance A is more commonly used in atomic absorption spectroscopy and is related to transmittance logarithmically as:

$$A = -\log T = -\log (I/I_0) \quad (2.8)$$

According to Beer's law, absorbance is directly proportional to the concentration c of the absorbing species in the flame:

$$A \sim c$$

Similarly, Lambert's law states that absorbance is proportional to the path length L of the sample cell aligned parallel to the light path:

$$A \sim L$$

Combining these two equations, we get the general Beer – Lambert's law:

$$A = K c L, \quad (2.9)$$

where K is the proportionality constant determined by the nature of the transition involved in the absorption and on the physical conditions such as temperature, pressure, and electrical fields, to which the atoms are subjected during the measurement.

Equation 2.9 shows that absorbance is directly proportional to both the concentration of the absorbing species and the path length. By plotting absorbance against concentration for a series of standard solutions, a calibration curve can be created, as illustrated below:

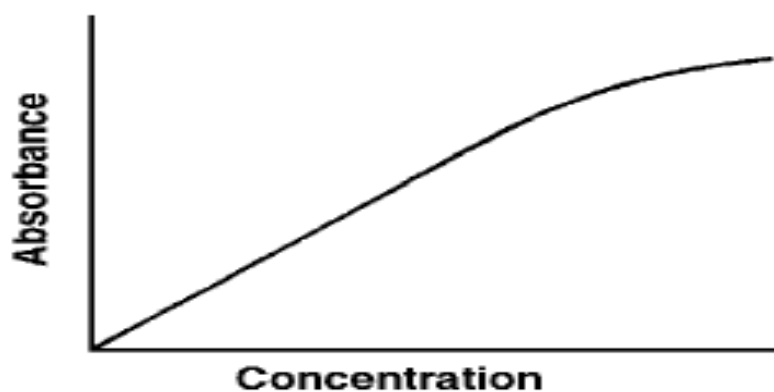


Figure 2.6: Graph of absorbance versus concentration illustrating Beer – Lambert's law (Lagalante, 1999).

This calibration curve can then be used to determine the unknown concentration of a sample by measuring its absorbance.

Overall, Beer-Lambert's law is crucial in FAAS spectroscopy for determining the concentration of metallic elements in a sample by using a calibration graph. (Mohammed, 2021; Currie, 1998).

2.3 Atomic Absorption Spectroscopy

Atomic Absorption Spectroscopy (AAS) is a widely used instrumental technique for analyzing more than 70 metallic elements in both organic and inorganic samples, detecting concentrations at the ppm (mg/L) or ppb ($\mu\text{g/L}$) level based on the atomization technique employed (Paudel, Kumar, & Mallil, 2021). Based on the atomization technique AAS is classified as: flame atomization, electrothermal atomization, glow discharge atomization, cold vapor atomization, and hydride atomization. This review will cover the history of AAS, atomic absorption theory, the principal components of AAS, its working principle, atomization techniques, and applications.

2.3.1 Short History of Atomic Absorption Spectroscopy

Different scientists starting from Wollaston to Alan Walsh has made their great contribution for the development and application of AAS to become a significant laboratory technique.

The phenomenon of atomic absorption was first observed by Wollaston in 1802 as dark lines in the solar spectrum. Brewster, in 1832, theoriatized that atomic vapor in the atmosphere absorbed some solar radiation, which led to the detection of these lines. Bunsen and Kirchhoff latter demonstrated that each chemical element emits characteristic spectrum when heated to

incandescence, confirming Wolaston's observations by reproducing the black lines in the solar spectrum in the laboratory. This enabled the identification of absorbing atoms through their absorption spectra.

In 1953, Alan Walsh developed AAS a powerful laboratory technique for determining trace metallic elements, making AAS a widely used method due to its high sensitivity, ease of operation, and low cost compared to other techniques (Lagalante, 1999). Today AAS is a powerful analytical tool for measuring metallic element concentrations by analyzing absorption spectra.

2.3.2 FAAS Instrumentation

The main components of an FAAS system are shown in the Figure 2.7. Which includes the light source, the nebulizer, spray chamber, burner, monochromator, detector, amplifier, signal processor, and display unit (Lagalante,1999).

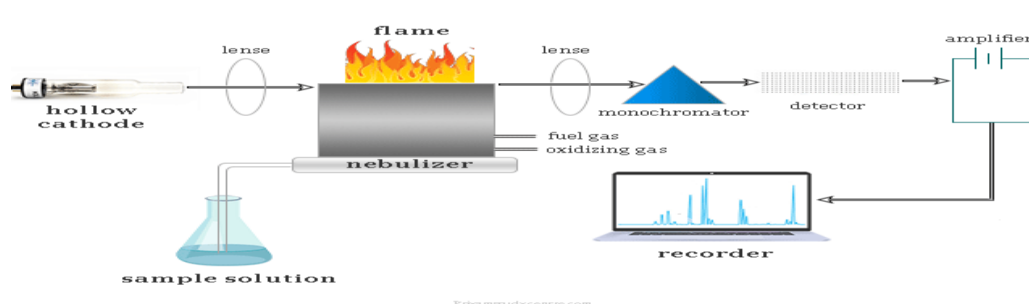


Figure 2.7: FAAS Schematic Diagram (Atomic absorption spectroscopy instrumentation, 2021).

2.3.2.1 Light Source in FAAS

In AAS, the light source emits radiation at specific wavelengths corresponding to the element being analyzed. The most commonly used sources are hollow cathode lamps, multi element hollow cathode lamps, and electrodeless discharge lamps.

A hollow cathode lamp, used extensively in AAS, works on the principle that ground-state atoms absorb light of characteristic wavelengths when in gaseous state. The lamp consists of cylindrical hollow cathode made of the element to be analyzed and a Tungsten anode, filled with an inert gas like Neon. When a voltage is applied, the gas atoms are excited and bombard the cathode, sputtering metal atoms that emit light at specific wavelengths as they return to their ground states. This emitted light passes through the quartz window and enters the sample cell, where the sample's atoms absorb the characteristic wavelengths (Lagalante,1999; The Perkin-Elmer Corporation,1999; Paudel et al., 2021).

2.3.2.2 The Burner System

The burner assembly in FAAS consists the nebulizer, spray chamber, and burner head. It produces ground-state atoms of via flame atomization for the detection process (Lagalante, 1999; Paudel et al., 2021).

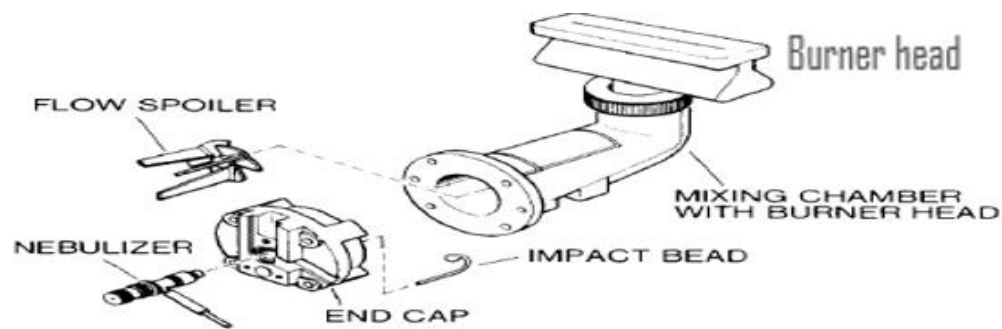


Figure 2.8: The burner assembly (Lagalante, 1999).

The burner system's role is to convert the samples solution into free atoms in gaseous form through flame atomization, where thermal energy vaporizes the sample and breaks chemical bonds to produce free ground state atoms. The analytical sensitivity of FAAS is largely dependent on the efficiency of this atomization process.

Nebulizer and mixing chamber.

Main steps involved to produce free atoms in gaseous form are: first we prepare a solution by dissolving our sample by appropriate solvent. Then the solution is sucked by a nebulizer to produce a fine spray of solution (aerosol). Then the aerosol from the nebulizer is led to the mixing chamber. The stream of liquid exiting the nebulizer collides with the impact beads, to shatter in to fine mist and mix with the nebulizer gases. Then the flow spoiler imparts rotational motion to the fine mist of aerosol to break into smaller more evenly distributed droplets and mixes with burner gas and fuel. The centrifugal force arising from rotation removes larger droplets, leaving only the smaller droplets to be sprayed into the flame by sprayer. Therefore, the spray chamber is highly inefficient with only 5% of the sample reaches the flame (Paudel et al., 2021; The Perkin - Elmer Corporation, 1999).

The Burner and Atomizer.

The slot burner, as shown in Figure 2.8, provides a long optical pathlength and a stable flame. Because absorbance is directly proportional to pathlength, a long pathlength provides greater sensitivity. A stable flame minimizes uncertainty due to fluctuations in the flame. The burner is mounted on an adjustable stage that allows the entire assembly to move horizontally and vertically. Horizontal adjustments ensure the flame is aligned with the instrument's optical path. Vertical adjustments change the height within the flame from which absorbance is monitored. This is important because two competing processes affect the concentration of free atoms in the flame. The flame serves as sample cell to hold free neutral ground state atoms. As the aerosol enters the flame (atomizer's) through the burner head, it undergoes three main processes:

- a) De-solvation (drying) - the solvent is evaporated and dry sample of nano particles remain.
- b) Vaporization - the solid particle is converted in to gaseous molecules.
- c) Atomization - molecules are dissociated into free atoms. Then, the free atoms within the flame are ready to be excited by a radiant energy emitted by cathode of the light source made up of the same element that is present in the sample.

2.3.2.3 Monochromator

Monochromator is an optical device that separates wavelength of light through dispersion (Mohammed, 2021). It consists of entrance slits, collimator (concave mirror to form parallel beam), diffraction grating (dispersive element), focusing mirror, and exit slit as shown in the figure bellow.

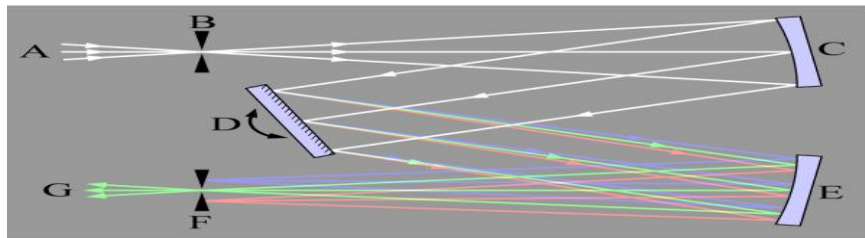


Figure 2.9: Czerny Turner monochromator (A- light entering monochromator, B- entrance slit, C- collimator mirror, D- diffraction grating, E- focusing mirror, F-exit slit, G- dispersed light ray) (Wikipedia).

In the figure shown UV and visible wavelengths enter the monochromator through an entrance slit where they are reflected onto the grating device where spectral separation occurs. The separated wavelengths were collimated with the focusing mirror towards the exiting slit. The rotation of the grating device determines the band of wavelength exiting the monochromator and reaching the detector.

The resolving power of a monochromator is governed by focal length, slit width, and rotation of the grating. Based on the type of element to be analyzed we have to optimize the monochromator parameters- slit width, focal length, grating angle. Therefore the monochromator selects and filter the spectral lines emerging from the hollow cathode lamp and allow passing only selected wave length towards the detectors (Paudel et al., 2021).

2.3.2.4 Detectors

Detectors convert the radiant energy into electrical signals; with photomultiplier tube being one of the most commonly used types (Paudel et al., 2021). As shown in the figure, it consists of photocathode, dynodes, and anode (collector). The Inner surface of cathode is coated with light sensitive material like Cs_2O , Ag_2O . The dynodes are coated with cesium metal which emits several electrons. These electrons are collected by collecting electrode (anode).

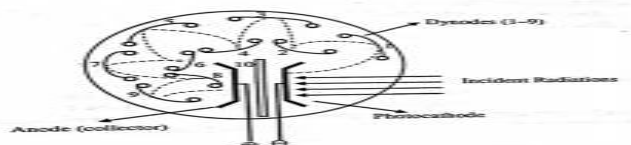


Figure 2.10: Photomultiplier tube (Wikipedia).

When light strikes on the cathode surface, electrons are ejected by photoelectric effect. These electrons strike on the surface of first dynode and ejection of 2 to 5 electrons take place. These electrons strike on surface of second dynode and ejection of more electrons take place. This process is continued up to the ninth dynode. Then the electrons are collected by collecting electrode and current begin to flow. This current is amplified and measured.

2.3.2.5 Amplifiers and Display Units

Amplifiers in Flame Atomic Absorption Spectroscopy (FAAS) are essential components that enhance the weak electrical signals generated by the detector, ensuring they are strong enough to be accurately processed and displayed. The primary role of an amplifier is to increase the signal's magnitude without altering its original information content. In a typical FAAS system, the amplified signal is proportional to the absorbance, which is related to the concentration of the analyte in the sample. The amplified signal is then sent to a signal processor or directly to display unit, where the absorbance can be read and interpreted (Paudel et al., 2021).

2.3.3 FAAS Working Principles

Atomic Absorption Spectroscopy (AAS) is a widely used analytical technique for detecting and quantifying metallic elements in both organic and inorganic samples, with detection capabilities reaching parts per million (ppm) and parts per billion (ppb) levels (Paudel et al., 2021). This method is particularly valuable in environmental monitoring, food safety, and clinical analysis due to its sensitivity and accuracy. FAAS, a specific type of AAS, operates by measuring the intensity of light absorbed by free gaseous metallic atoms in a flame, which correlates with the concentration of the target element in the sample.

The FAAS process begins with sample preparation, followed by the creation of calibration curve, which plots light intensity against concentration. The sample is then introduced into the flame via the nebulizer, where it is atomized to produce free ground-state atoms. These atoms absorb light emitted by a hollow cathode lamp specific to the analyte. The absorption of light causes the atoms to transition from ground state to excited state, corresponding to the energy difference between these levels.

A monochromator, positioned between the flame and the detector, isolates the specific wavelength of interest, thereby minimizing background interference. The detector measures the absorbed light intensity and converts it into an electrical signal, which is subsequently amplified and processed. The final output, displayed as an absorbance spectrum, provides quantitative information about the element's concentration based on the calibration curve.

One of the key advantages of FAAS is its cost-effectiveness and ease of operation, making it accessible for routine analysis. It offers good sensitivity, with the capability to detect many elements at ppm level or lower, and it provides high precision and accuracy through calibration curves with minimal inter-elemental interference (Paudel et al., 2021). However, FAAS also has limitations, such as reduced sensitivity due to spectral noise from the flame and the requirement to measure one metal at a time, necessitating different lamps for each element analysis. Furthermore, the atomization process is inefficient, with only about 5% of the sample converted to aerosol, leading to lower detection limits. Additionally, FAAS is not suitable for nonmetals (Lagalante, 1999; The Perkin-Elmer Corporation, 1999).

2.4 Instrumental Methods of Heavy Metals Analysis

The determination of the concentration of unknown analyte in various environmental samples involves several key steps, including sample preparation, instrument calibration, and method validation using appropriate quality assurance and control techniques.

2.4.1 Sample Preparation

Proper Sample preparation is crucial for accurate heavy metal analysis using flame atomic absorption spectroscopy (FAAS). The steps involved in preparing bottled water samples for analysis include:

- a) Selection and Collection: Choosing and collecting bottled water samples using proper sampling technique to ensure the accurate analysis of heavy metals.
- b) Sample Homogenization: If the bottled water has been stored for an extended period, it may require homogenization.
- c) Preservation: Treating (acidifying) the water with nitric acid to preserve heavy metals, which helps minimize precipitation and adsorption onto glass containers walls. Alternatively, digestion with concentrated nitric acid can be performed to oxidize organic matter present and release heavy metals.
- d) Dilution: After treatment, diluting the sample with distilled water ensures that the concentration falls within the linear detection range of the instrument.
- e) Filtration: Filtering the diluted sample using appropriate materials remove any particulate matter and impurities, making it ready for the analysis.

Effective sample preparation technique enhances sensitivity and improves detection limits, leading to more accurate results (Bader, 2011; Smith, 1983; Welna, Anna & Pohl, 2011).

2.4.2 Instrument Calibration

Instrument calibration involves establishing a mathematical relationship between the element concentration and the instrument's response (Julian, 1984). Calibration typically follows four

steps: preparing standard solutions, optimizing instrumental parameters, measuring the analytical signal, and using mathematical calculations to determine function model.

Standard solutions are prepared by diluting concentrated stock solution. These solutions, along with an analytical blank, are analyzed to plot the element concentration against the instrument's response. The concentration of an element in a sample is then determined using interpolation. The instrumental response versus concentration is plotted by linear equation as:

$$Y = mx + b \quad (2.10)$$

Where Y absorbance signal, m slope, and b y - intercept.

The linearity of the instrumental response is statistically demined by using correlation coefficient (r). If r = 1 then there is a strong correlation between absorbance and concentration (Julian, 1984; Shaltout, & Ibrahim, 2007; Miller, J. N and Miller, J. C. ,2010).

2.4.3 Validation Techniques

Validation ensures that the analytical method is suitable for its intended purpose (Bader, 2011). It involves quality assurance and control methods.

Sample preparation methods contribute to 30 % of the total error and consume 60% of the total experimental time (Welna et al., 2011; Nabil, B. 2017). Therefore, implementing proper sample preparation techniques based on analytical protocols essential to avoid contamination, minimize analytical losses, and reduce interferences.

Accurate results depend not only on proper sample preparation but also assessing the instrument's limitations through quality control parameters such as correlation coefficient,

accuracy, precision, and detection limits (Welna et al., 2011; Miller et al.2010). In general, to obtain accurate and reliable results the quality assurances and control parameters should be implemented.

2.5 Application of AAS

Atomic absorption spectrometry (AAS) is a versatile analytical technique used to measure concentrations of 70 elements in various samples, with sensitivity down parts per billion (ppb) (Mohammed, 2021). This versatility makes AAS applicable across multiple fields, including mining, pharmaceuticals, environmental control, agriculture, beverage production, and forensics investigations. The technique's ability to provide both qualitative and quantitative data ensures its critical role in safety and compliance across these sectors

2.5.1 Pharmaceutical application

AAS is extensively used in pharmaceutical quality control to ensure drugs are free from residual catalysts, such as palladium or platinum, which are used during production (Rmesett et al, 2010). For instance, Nageswara et al., (2010) used AAS to analyze the concentration of both heavy metals (Pb, Cd, Hg, and Cr) and essential metals (Zn, Mn, Ni, Co, Cu and Fe) in various medicine formulations, including allopathic (paracetamol and diethazine hydrochloride) and ayurvedic (Mruthyunjaras) products. The medicines were purchased from different pharmacies, and 1g of the powdered samples was digested with 5 ml of concentrated HNO₃ at 80°C for 10 min in 100ml beaker. After cooling, the solutions were filtered and diluted to 100ml. The AAS instrument was calibrated with standard solutions, and the method was validated for accuracy, precision, and linearity. The result revealed that cold

medicines contained higher levels of toxic and essential metal contaminants than WHO limits, with Cr, Hg, Pb, and Fe exceeding permissible levels, highlighting the importance of continuous monitoring (Nageswara et al., 2010).

2.5.2 Mining Application

AAS plays a vital role in mining, particularly in quantification of precious metals like gold. Fouad et al. (2015) employed FAAS to determining gold concentrations in organic samples by optimized instrumental and chemical parameters, such as burner angle, fuel flow rate, band pass, burner height, media selection, leaching and interference effects. Their method enabled the direct determination of gold in the organic phases, and validation with certified standard solutions showed a percentage error between 1.5% and 2.5 % (Fouad et al., 2015).

2.5.3 Beverage Application

The beverage industry uses AAS to monitor heavy metal concentrations, ensuring product safety. Alkis et al. (2014) analyzed the concentration of heavy metals in 43 wine brands (37 red, and 6 white) from four Turkish regions using AAS with electro thermal atomization techniques. Samples were treated with hot HNO₃ and H₂O₂ to decompose the organic matrix, and the instrument was calibrated with detection range 1 – 200 μ g/L. Certain heavy metals such as Cr, Mn, Fe, Co, Ni, Cu, Zn were detected, but their concentration were below WHO limits. Cd and Pb were found in red wines but were undetectable in white wines. Accuracy was confirmed through the standard addition method, with recoveries ranging from 96% to 107% (Alkis et al., 2014).

2.5.4 Forensics investigation application

In forensic science, AAS is used to link soil samples to specific locations based on their elemental composition. Maurya et al. (2018) used AAS to investigating the relation between land use type with of heavy metal concentrations in soils to characterize soil. Elemental composition and concentration of a soil can be used as fingerprint. For studying, soil samples were collected from river sites, industrial areas, urban regions, and institutional areas, and were digested by using HCl and HNO₃. The study found higher concentrations of Zn, Mn, and intermediate levels of Cd and Ni. The result demonstrated that each sample had a unique heavy metal concentration profile, serving as a “fingerprint” for identifying pollution source (Maurya et al., 2018).

Since 1960s, AAS has become a crucial tool for qualitative and quantitative analysis in different fields due to continuous advancements in technology.

2.6 Heavy Metals in Bottled Water

In this section provides an overview of heavy metals and summarizes research on their presence in bottled water.

2.6.1 Heavy Metals

Heavy metals are naturally occurring elements that can enter drinking water systems through both man-made or natural process, posing significant public health risks when concentrations exceeded specific limits.

Heavy metals, including metalloids, have specific densities five times greater than that of water (Tchounwou et al., 2012; Luqueño et al., 2013; Durube et al., 2007). Examples include

mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), silver (Ag), and zinc (Zn). While some of these metals, such as iron, cobalt, copper, manganese, molybdenum, and zinc, are essential micronutrients for metabolic functions, they become toxic at concentrations above the limits set by regulatory authorities. Others, such as lead, mercury, cadmium, and silver, are non-essential and toxic even at low concentrations (Luqueño et al., 2013; Das et al., 2011; Durube et al., 2007).

Heavy metal pollution in drinking water can result from manmade activities such as industrial activities, mining, landfills, waste disposal, industrial discharges, traffic emissions, pigment manufacturing, battery and cement plants, refineries, electronics manufacturing, pesticide uses, fertilizers, and herbicides used in agriculture. And the natural source that leads to heavy metal pollution of the drinking water are associated to the natural occurrence of these metals on the earth's crust. Weathering of sedimentary rocks, volcanos, geochemical conditions, geothermal sources, and microbial activities leads to heavy metal pollution of drinking water and when consumed affects the health of the public (Tchounwou et al., 2012; Luqueño et al., 2013; Das et al., 2011; Durube et al., 2007).

Exposure to high concentration of heavy metals in drinking water can lead to serious health problems, including damage to the brain and the central nervous systems that leads to paralysis, damages the lung tissues and affects the respiratory system, whereas some accumulates in the internal organs and affects the cells and organelles that leads to the development of cancer. Long-term exposure to heavy metals may result in a slowly progressing physical, muscular, and neurological degenerative effects that results Alzheimer's

disease, Parkinson's disease and muscular dystrophy (Tchounwou et al., 2012; Luqueño et al., 2013; Das et al., 2011; Durube et al., 2007). Common health metals of health concern includes like Nickel, Coper, chromium, cobalt, and lead.

Nickel (Ni): is essential micronutrient, but excessive exposure can have adverse effects on gastrointestinal, hematological, neurological and immune systems (Tchounwou et al., 2012). Nickel contamination in bottled water can occur from Nickel- chromium plated fittings, stainless steel pipes, welding products, or from the water source and any treatment applied (WHO, 2021). Most likely, the contamination can be related with the nature of the geology of the aquifer like depth of drilling, and nature of the rock layers through which the spring passes (Ungureanu et al., 2022).

Coper (Cu): It is micro nutrient essential for physiological functions like hemoglobin synthesis, melanin formation, phospholipid synthesis, and collagen synthesis (Tchounwou et al., 2012). However, high levels of copper in drinking water can lead to chronic anemia, parkinsonism, cognitive disfunction, and Alzheimer's disease (Luqueño et al., 2013).

The contamination of bottled water with copper may be due to variations in water characteristics like pH and hardness, as well as corrosion of copper piping systems (Toma, 2011). Or it can be related to the characteristics of the rock layers through which the spring passes (Ungureanu et al., 2022).

Chromium (Cr): is naturally present in rocks, soils, plants, and animals and can existed in solid, liquid, or gas (Das et al., 2011). It is an essential micronutrient for glucose, fat, and protein metabolism, but it is toxic at high concentrations particularly in its hexavalent form.

Chronic exposure to chromium can cause skin ulceration, dermatitis, and long-term exposure can lead to liver, kidney, circulatory and nerve tissue damage (Tchounwou et al., 2012).

Chromium contaminations in the bottled water samples analyzed may have originated from the corrosion of the stainless-steel storage material, chromium compounds used in stainless steel welding, chrome plating as anticorrosive in water storage tankers, or from natural source (Tchounwou et al., 2012).

Cobalt (Co): It occurs in the environment in association with other metals such as copper, nickel, manganese, and arsenic. Its levels in water bodies can increase due to anthropogenic activities such as application of cobalt-containing phosphate fertilizers and the burning of fossil fuels (Durube et al., 2007).

Cobalt is an essential micronutrient as it forms part of vitamin B₁₂ and is used for treatment of anemia, however long-term exposure to cobalt can cause gastrointestinal effects, liver injury, and allergic dermatitis (Tchounwou et al., 2012).

Lead (Pb): Lead and its compounds are widely spread in the environment due to human activities, such as fossil fuel burning, mining, and manufacturing (Das et al., 2011). Lead is a systemic toxicant that affects several organs, including the kidney, liver, central nervous system, and endocrine system. Acute lead exposure can cause kidney damage, and gastrointestinal diseases, while chronic exposure can result in severe damage to the central nervous system, kidney, blood pressure, and vitamin D metabolism (Tchounwou et al., 2012; Durube et al., 2007).

Lead contamination in bottled water can occur from the plumbing components, service pipes, and lead based solders used to join joints in the pipe lines (Hu et al.,2018) or it may be associated with the geology of the aquifers (Olowoyo et al., 2022).

In general, these heavy metals can enter the human body through drinking water. One of the drinking waters that is available now a days is bottled water. Bottled waters can be sourced from ground water or from springs. Therefore, heavy metals leak to these sources due to natural or man-made activities (Luqueño et al., 2013; Durube et al., 2007).

2.6.2 Literature Review on Contamination of Bottled Water by Heavy Metals

Heavy metal contamination in bottled water is a significant global public health concern (Luqueño et al., 2013). Numerous studies using various spectroscopic techniques have been conducted worldwide to assess the presence of heavy metals in bottled water. Below is a review of some of the key studies that have employed different spectroscopic methods to determine the levels of heavy and common metals in bottled drinking water.

Tolera Seda et al. (2013) investigated the presence of common metallic ions and trace metals in nine brands of bottled mineral water consumed in Addis Ababa, Ethiopia. They measured the concentration of heavy metals and trace metals in these samples using a FAAS buck scientific model 201 VGP equipped with deuterium background correction. Some common metals were detected but trace metals were not detected, except iron and zinc in certain brands of bottled mineral water (Seda, T. et al., 2013).

Omeje Maxwell et al. (2018) analyzed the heavy metal content in twenty different brands of bottled water samples collected from various shops in Lagos, Nigeria. The concentration of

heavy metals was determined by using Graphite Furnace Atomic absorption spectrometer connected to win lab 32 software, they detected cadmium, lead, nickel, and iron. Their findings indicated that four of the bottled water samples were significantly contaminated, exceeding WHO standards. The accuracy of their methods was validated by calibration curves with a value close to one (Mawell, O., et al., 2018).

Michael J. Sullivan (2011) conducted a study to identify seventeen heavy metals in six sample of bottled natural spring water available in markets of Californian, USA. thirteen different heavy metals were detected in the samples to $\mu\text{g/L}$ bellow the WHO permissible limits (Sullivan, 2011).

Olowoyo, J.O et al. (2022) determined the concentration of twelve heavy metals in twelve bottled water samples purchased from supper market in Pretoria, South Africa using FAAS technique. Their results reveled, that concentrations of chromium, lead, and nickel in the water samples exceeded the WHO limits while other metals such as iron, copper, manganese, vanadium, titanium, molybdenum, and cadmium were detected at levels below WHO standards (Olowoyo et al., 2022).

Moges Mitiku & Lake Indeshaw (2022) conducted an analysis on a sample of six branded bottled mineral waters widely available in the Ethiopian market. The samples were prepared using hot plate digestion by boiling 100 ml of water with 1.5 ml of HNO_3 until the volume reduced to 20 ml. Additional 1.5 ml of HNO_3 was then added and heated until a clear solution was obtained, indicating that digestion was complete. The instrument was calibrated by diluting a stock solution, and the concentration common metallic ions such as potassium, sodium, zinc, iron, calcium, and magnesium was determined using FAAS. Their findings

were compared with the labels on the bottles, showing slight variations but remaining within WHO limits (Mitiku, & Endeshaw, 2022).

Contamination of bottled water can occur due to the packaging material, particularly plastic bottles made from polyethylene terephthalate (PET) and recycled PET (R-PET) (Shotyk & Krachler, 2007). Heavy metals associated with pigments such as Pb, Cd, or Cr, and catalysts residues such as antimony trioxide (commonly used in PET production), as well as cadmium-based stabilizers in polyvinyl chloride (PVC) can contribute to contamination. Additional factors like prolonged storage at high temperatures, solubility of migrants, and the degree of plasticization can exacerbate this contamination (Ungureanu et al., 2022; WHO, 2004; Olowoyo et al., 2022).

The contamination may also occur during the bottling process due to infrastructure corrosion. Heavy metals can leach into water from aging or corroded infrastructure, including pipes, storage tanks, and plumbing system (WHO, 2021).

Mitku and Endeshaw (2022) demonstrated a strong positive correlation ($r > 0,99$) between soil parameters where the water is sourced and the heavy metal content in bottled water. Since most bottled water is sourced from boreholes, there is a direct relationship between the mineral-rich soil and the heavy metal content in the bottled water (Olowoyo et al., 2022).

From these reviews, it is evident that Atomic Absorption Spectroscopy (AAS) has been widely used to determine the concentration of heavy metals using either flame or graphite atomization techniques. studies conducted world have consistently shown the presence of heavy metals in bottled water. However, research in Ethiopia has primarily focused on determination of common ions and physiochemical parameters of bottled mineral water in

limited geographic area without considering heavy metals. Additionally, there is no information regarding heavy metals on the labels of the bottled water.

This research was therefore initiated to determine the concentration of heavy metals in various brands of bottled drinking water consumed in Ethiopian market. The study involved collecting sufficient number of samples covering different geographic locations across the country and utilizing the flame atomic absorption spectroscopic technique to its detection limit. Proper optimization techniques were implemented according to manufactures specifications.

CHAPTER THREE

MATERIALS AND METHODS

This chapter details the materials and methods used in this research to analyze the concentrations of selected heavy metals (Pb, Ni, Cr, Cu, and Co) in bottled water brands available in Ethiopian markets. The chapter is structured first, to describe the materials and equipment employed, followed by the procedures for sample collection and preparation. Subsequently, the analytical methodologies applied to determine heavy metal concentrations, along with validation and data analysis techniques, are thoroughly discussed. The methods are selected to meet the research objectives.

3.1 Materials

The materials utilized in this research are critical to ensure accurate and reliable analytical results, which are foundational to achieving the study's objectives. The primary instrument used for the analysis was a flame atomic absorption spectrometer (FAAS), specifically the BUCK SCIENTIFIC MODEL 210 VGP, selected for its sensitivity and precision in detecting trace levels of heavy metals in aqueous samples. Supporting laboratory tools including volumetric flasks, funnels, measuring cylinders, and pipets, all of which were properly prepared to avoid contamination.

The chemical reagents including 2% nitric acid used to acidify the samples and prevent metal precipitation or adsorption onto the container walls. Calibration of the FAAS was performed using, 1000 mg/L stock standard solutions of the target (Pb, Ni, Cr, Cu, and Co), and distilled water was used to minimize contamination and for dilution.

3.2 Study Area and Sample Selection

3.2.1 Geographic Scope

Presently more than 106 brands of bottled waters are available in Ethiopian market that are produced and bottled by different bottling companies located in different regions of Ethiopia (Yewondwossen, M. 2022, February 7). For study purpose, the sampling areas were clustered in to four zones: north, central, south, and west Ethiopia. The North Ethiopia includes east and west Amhara region; central Ethiopia includes Addis Ababa and central Oromia region; south Ethiopia that includes Sidama region, and North East Oromiya region: and the west Ethiopia includes west Oromia region round Gimma.

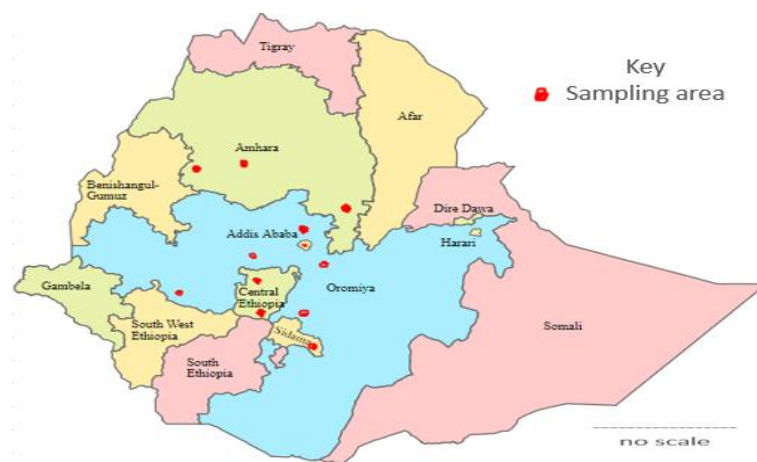


Figure 3.1: Geographic location of sampling area indicated by red spots.

3.2.2 Sample Selection Criteria

From each clustered zone, at least four brands of bottled water, with capacities ranging from 0.5 to 2 liters, were randomly selected, resulting in a total of 18 different samples collected from September to July 2016 E.C. The samples were purchased from local shops and stored at 4 ° c in their original sealed container until the analysis.



a, Collected samples.

b, In chemistry laboratory.

Figure 3.2: Collected bottled water samples from Ethiopian markets.

Table 3.1: Description and the source area of bottled water used in the present study.

Brand cod	Brand name	Description of water type	Source area and region
BW-1	Africa water	Purified ground water	Holota – Oromiya region
BW-2	Gift water	Purified natural water	Sululta –South west Oromiya region
BW-3	South water	Purified spring water	Shashemene– Oromiya region
BW-4	Tseday water	Natural spring water	Gurage mountain- Meakelawi Ethiopia region
BW-5	Aqua Gimma	Natural purified water	Jimma – West oromiya region
BW-6	Gold water	Purified natural water	Tatek – oromiya region
BW-7	Fiker water	Natural spring water	Gurage mountains, meakelawi Ethiopia region
BW-8	Sheger water	Natural purified water	Sebeta , oromiya region
BW-9	Selam water	Natural spring water	Shashemene, South oromiya region
BW-10	One water	Purified natural water	Mogole mountain, Ambo zone, west oromiya region
BW-11	Choice water	Purified natural water	Bahrdar, Amhara region
BW-12	Telil water	Natural spring water	Siltezone,,Meakelawi Ethiopia region
BW-13	Siket water	Natural purified water	Dangla zone, East Amhara region
BW-14	Aman water	Purified natural water	Welliso, south west oromiya region

BW-15	Dear water	Purified ground water	Nefas silk, Lafto sub-city Addis Abeba
BW-16	Beza water	Natural spring water	Aleta Wondo, Sidama region
BW-17	Aqua sef	Purified natural water	Debrebrhanm East Amhara region
BW-18	Dangla water	Natural spring water	Awi zone West Amhara region

3.2.3 Laboratory Sample Preparation Technique

Sample preparation technique is the main activity that is performed next to sample collection to analyze heavy metals since it takes 61% of our analytical time and accounts 30% of the total error (Bader, 2011; Nabil, B. 2017).

In this research, for heavy metal analysis we used the following methods for each brand of bottled water. This includes flask cleaning, sample preparation, sample treatment.

3.2.3.1 Flask Preparation

Flask preparation is the first step in analytical method that is done to avoid contamination that comes from previous sample remains in the container for the sake of quality assurance (Bader, 2011).

In this research to avoid contamination all the glass wares and apparatuses used throughout the analysis process were first washed with tap water and detergents. Next rinsed with distilled water repeatedly. Then it is soaked in 10 % nitric acid for twelve hours. Finally, the acid is removed and rinsed with distilled water repeatedly and then air dried to make it ready for sample treatment step.

3.2.3.2 Sample Treatment

Three different water samples were taken directly from each sealed bottled water, ensuring no prior contamination, in a separate container. The samples of the bottled water were treated

(acidified) with 2% nitric acid to reduce the pH < 2 and to preserve the sample by preventing the precipitations and adsorption on glass containers and to remove organic matters if present before FAAS analysis. So that 2 ml (2% HNO₃) was added to 100 ml of bottled water sample and left for one hour and then ready for the analysis. This method is straightforward and quick, requiring minimal reagents and preparation time, and low risk of contamination since it involves fewer steps (Bader 2011; The Perkin - Elmer Corporation ,1999; Smith, 1983; Welna,M. et al., 2011).

3.3 Instrumentation and Calibration

3.3.1 Instrumental Set Up

A state-of-the-art flame atomic absorption spectroscopy FAAS (BUCK SCIENTIFIC MODEL 210 VGP) equipped with hollow cathode lamps for each heavy metals (Co, Cu, Ni, Pb, and Cr) was used to determine the concentration of Pb, Ni, Cr, Cu, and Co.

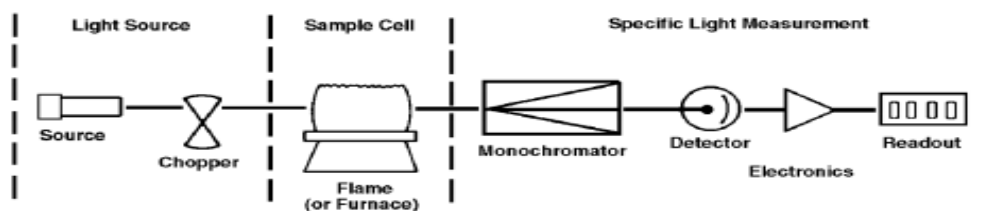


Figure 3.3: Schematic diagram of FAAS setup.



Figure 3.4: FAAS set up in Laboratory.

3.3.2 Instrumental Parameters

The instrumental parameters: resonance line, band pass, lamp current, flame type, fuel flow rate, air flow rate must be optimized to enhance sensitivity and reduce interference (Julian, 1984; Currie, 1998).

The optimized parameters that were used in this research includes the most sensitive resonance line, slit width, lamp current, and energy of radiation. All these are determined through iterative optimization methods. The data for these parameters was shown in table 3.2.

Table 3.2: Optimized instrumental working parameters of FAAS for selected heavy metals.

Heavy metals	Wave length (nm)	Slit 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	217.0	0.7	2.0	2.874	0.004
Co	240.7	0.2	4.5	3.106	0.050

3.3.3 FAAS Calibration

Instrument calibration in atomic absorption spectroscopy is done by step wise dilution of concentrated stock solution to produce a series of standard working solution with known concentrations (Tyson, J., et al.,1984). 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 obtain working standard solutions to calibrate the instrument (Smith, R., 1983)

In this study for the determination of heavy metals a series of six standards including calibration blanks (s_0) such as (s_1, s_2, s_4, s_5, s_6) were prepared for each heavy metals from their

working standard solution (10mg/l) in different calibration range by diluting with deionized water. Then the standard solutions were run sequentially and the absorbance signal at the selected wave length was measured for each concentration to establish a calibration curve. Calibration curves for each selected metal was set to ensure the accuracy of the instrument and to confirm that the results of determination were true and reliable. The quality of results obtained for heavy metal analysis using FAAS are seriously affected by the calibration and standard solution preparation procedures. Calibration standards for the elements analyzed were prepared in concentration range expected for the analyte in the samples analyzed (Smith, R., 1983; Tyson, 1984).

In this study for background signal correction the absorbance signal value for a blank was subtracted from the absorbance signal values of the standards. Then we plot a calibration curve using the working standard concentrations and their corresponding absorbance values. Then, the curves serve as a reference for quantifying unknown sample concentrations. The calibration standards for each heavy metal are shown in the table 3.3 below.

Table 3.3: Calibration standard for heavy metals.

Heavy Metals	Concentration Of heavy Metals(mg/l)				
	S₀	S₁	S₂	S₃	S₄
Pb	0.00	0.05	0.1	0.5	1.0
Ni	0.00	0.05	0.1	0.5	1.0
Cu	0.00	0.01	0.05	0.5	1.0
Cr	0.00	0.05	0.5	1.0	2.0
Co	0.00	0.05	0.5	1.0	2.0

3.4 Method Validation

Analytical method validation refers to the evaluating and proving the quality of an analytical method used for determination of heavy metals in relation to the standard protocol. Also, it assures that the selected method will give reproducible and reliable results (Welna et al., 2011; Miller et al., 2010). In this research the validation methods used were linearity test, limit of detection, accuracy and precision.

3.4.1 Linearity Test

Linearity test refers to the ability of analytical procedure to produce accurate and reliable results in direct proportion to the concentration of analyte in sample within the required concentration level (Miller et al., 2010).

In this research the linearity test was performed by measuring the absorbance of a blank and standard solutions of heavy metals in their working range. Based on the data absorbance curve was plotted and correlation coefficient was determined. The acceptance criteria for the correlation coefficient are ≥ 0.999 (Miller et al., 2010).

3.4.2 Instrumental Detection Limit

The instrumental detection limit (IDL) is the lowest concentration of analyte that can be detected and distinguished from zero. For FAAS IDL was calculated based on the standard deviation of the response (absorbance) and slope of calibration curve using the equation:

$$IDL = 3\sigma / S, \quad (3.1)$$

where σ = the standard deviation (error) of the response (absorbance signal), and S = slope of the calibration curve (International Conference on Harmonization (ICH), 1994; Miller et al., 2010).

3.4.3 Precision

It describes the closeness of values in a measurement. It describes the reproducibility and repeatability of the measurement (International Conference on Harmonization (ICH), 1994).

For our study we used only the reproducibility test. It accounts only the error coming from the operating system and not from error attributed to sample handling. It was carried out by taking three readings during measuring the absorbance of each analyte in the sample and measured as relative standard deviation (%RSD) using equation:

$$\%RSD = (SD / \text{mean value}) \times 100 \% \quad (3.2)$$

According to RSD Horwitch function (Gonzalez and Herrador, 2007), the maximum RSD values acceptable for the level of concentration up to 1ppm (1mg/L) is 16%.

3.4.4 Accuracy

Accuracy is the measure of how close an experimental value is to the true value. It can be performed by using standard reference materials or by recovery test for the spiked samples. The acceptable range for recovery typically falls between 80% and 110% for a concentration up to 1ppm (1mg/L) (Gonzalez et al., 2007). We conducted recovery tests for our analysis by adding a known amount of each heavy metal's concentration to a sample. It is calculated by using equation:

$$\text{Recovery } (\%) = [(\text{Measured concentration in spiked sample} - \text{Measured concentration in control sample}) / \text{Amount spiked}] \times 100\% \quad (3.3)$$

3.5 Data Analysis and Representation

Qualitative analysis was used to identify the presence of specific heavy metals, while quantitative data obtained from FAAS technique were analyzed by descriptive statistics including mean concentrations and standard deviation for each heavy metals across the different brands by using Microsoft excel. The results were presented through tables and bar graphs providing a clear and comprehensive description of heavy metals concentrations in the bottled waters

3.6 Comparison Parameters

The heavy metals concentrations determined in this study were compared and related with national and international drinking water quality standards, this includes WHO, and Ethiopian quality standards. Additionally, the results were compared with those from similar studies in other countries to contextualize the level of contamination in Ethiopian bottled water. This comparison provides crucial insights in to the safety of bottled water and highlights any potential health risks associated with heavy metal contamination.

In general, the methodologies employed in this study were carefully selected and validated to ensure the accuracy of heavy metal concentrations in bottled water samples. By implementing strict quality control measures such as linearity test, detection limits, precision test, and recovery test. The study provides reliable data that that can inform regulatory standards and public health recommendations. The comparison with WHO and other international standards further underscores the significance of the findings and the need for continued monitoring of bottled water quality in Ethiopia

CAPTER FOUR

RESULTS AND DISCUSSIONS

In this chapter, the results and discussions of the research are presented. Section 4.1 displays the concentrations of detected heavy metals in different bottled water brands through tables and bar graphs. Section 4.2 discusses the findings in comparison with national and international guidelines, and with related studies. Finally, Section 4.3 Covers the validation of methods using techniques like linearity test, detection limits, precession, and accuracy against acceptable standard criteria.

4.1 Results of Heavy Metals in Bottled water Samples

The selected heavy metals (Lead, Chromium, Nickel, Copper, and Cobalt) were analyzed using a validated FAAS spectroscopic method. All measurements were conducted in triplicate, with the results presented as the mean of the replicated measurements \pm standard deviation. The results are shown in table 4.1.

Table 4.1: Average concentrations (mean \pm SD) of heavy metals concentration in bottled water samples in ppm (mg/L).

Brand name	Brand ID	Ni (mg/L)	Cu(mg/L)	Cr (mg/L)	Co (mg/L)	Pb (mg/L)
Africa	1	BDL	0.028 \pm 0.002	BDL	BDL	0.0073 \pm 0.0006
Gift	2	0.053 \pm 0.002	0.021 \pm 0.001	BDL	BDL	0.0064 \pm 0.0001
South	3	0.046 \pm 0.003	BDL	0.049 \pm 0.002	BDL	0.0082 \pm 0.0001
Tseday	4	0.068 \pm 0.002	0.075 \pm 0.003	0.044 \pm 0.004	BDL	0.0061 \pm 0.0001
Aqua Gim	5	0.054 \pm 0.003	0.064 \pm 0.001	BDL	BDL	0.0057 \pm 0.0001

Gold	6	BDL	0.084 ± 0.004	0.045 ± 0.002	BDL	0.0050 ± 0.0000
Feker	7	0.044 ± 0.002	BDL	BDL	BDL	0.0083 ± 0.0006
Sheger	8	BDL	0.054 ± 0.003	BDL	BDL	0.0053 ± 0.0005
Slam	9	0.023 ± 0.002	0.025 ± 0.001	BDL	BDL	0.0063 ± 0.0006
One	10	BDL	0.037 ± 0.003	0.046 ± 0.003	BDL	0.0063 ± 0.0004
Choice	11	0.021 ± 0.002	0.04 ± 0.003	BDL	BDL	0.0054 ± 0.0003
Telil	12	0.022 ± 0.001	0.037 ± 0.002	BDL	BDL	0.0070 ± 0.0006
Sket	13	BDL	0.054 ± 0.002	0.049 ± 0.004	BDL	0.0064 ± 0.0006
Aman	14	0.035 ± 0.003	BDL	BDL	BDL	0.0053 ± 0.0005
Dear	15	BDL	0.045 ± 0.003	BDL	BDL	0.0093 ± 0.0004
Beza	16	0.045 ± 0.001	BDL	0.049 ± 0.002	BDL	0.0050 ± 0.0000
Dangla	17	0.054 ± 0.002	0.054 ± 0.002	0.049 ± 0.002	BDL	0.0057 ± 0.0006
Aquasef	18	0.056 ± 0.002	0.055 ± 0.0001	0.048 ± 0.003	BDL	0.0056 ± 0.0004

Table 4.2: Current drinking water quality guidelines (mg/L) for heavy metals published by international and national organizations.

Standards	Heavy metals				
	Chromium	Copper	Lead	Nickel	Cobalt
WHO ^a	0.05	2	0.01	0.07	-
ETH ^b	0.05	2	0.01	0.07	-

^a, World Health Organization (WHO 2017); ^b, Ethiopian Guide Line (2002); - limit not set.

4.1.1 Result Analysis by Heavy Metal

4.1.1.1 The Concentration of Nickel (Ni) in the Bottled Water Samples

Nickel was detected in 12 bottled water samples, with concentration ranging from lowest (0.021 ± 0.002) mg/L in Choice bottled water to highest (0.068 ± 0.002) mg/L in Tseday

bottled water sample as presented in figure (Figure 4.1) and table (Table 4.1). Comparison with WHO's and Ethiopian's maximum permissible limit is presented in figure (**Figure 4.2**).

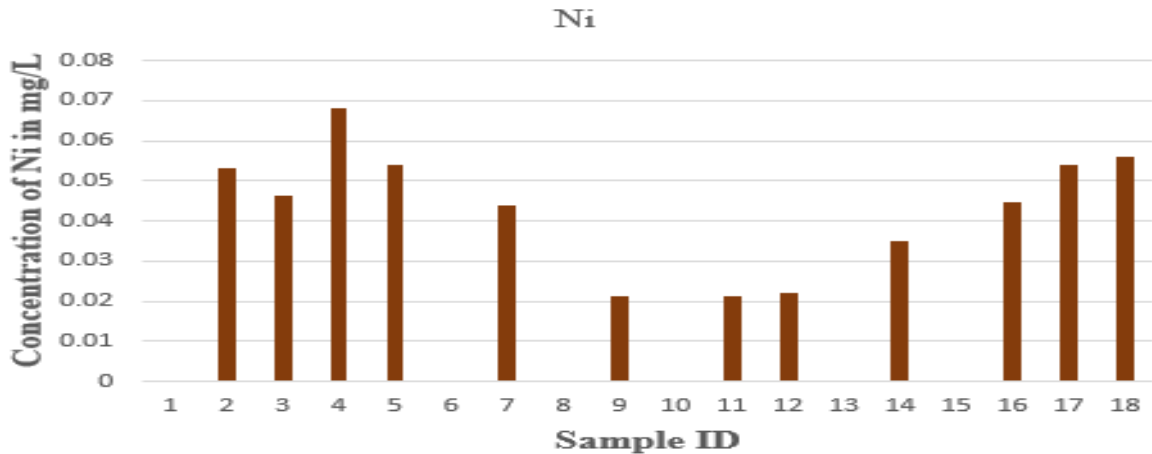


Figure 4.1: The average concentration of Nickel (Ni) against samples ID in the bottled water samples.

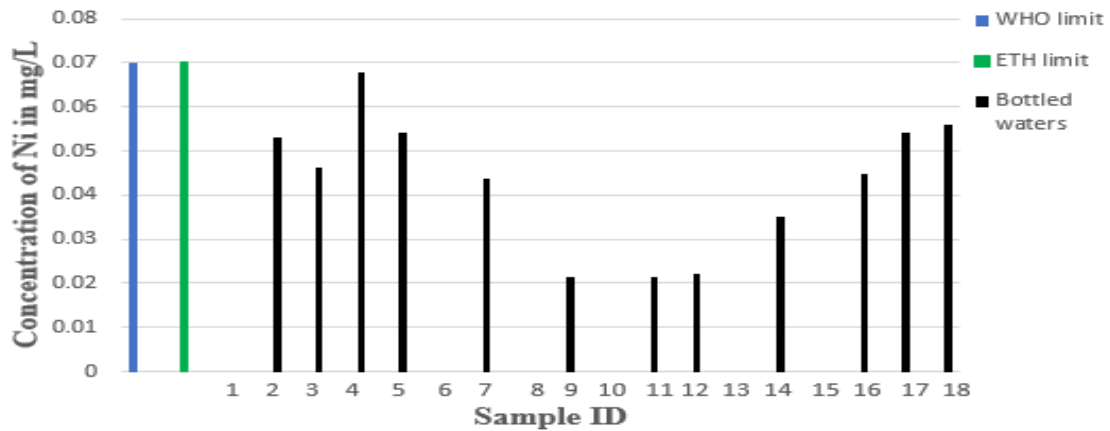


Figure 4.2: The average concentration of Nickel (Ni) against samples ID and international standards.

4.1.1.2 The Concentration of Copper (Cu) in the Bottled Water Samples

Copper was detected in 14 bottled water samples. The result of the study showed that concentration of copper varies from the lowest (0.021 ± 0.001) mg/L in Gift bottled water sample to the maximum of (0.084 ± 0.004) mg/L in Gold bottled water sample as presented in

figure (Figure 4.3) and table (Table 4.1). The comparisons of the values obtained from this study with national and international guide line is presented in **Figure 4.4**.

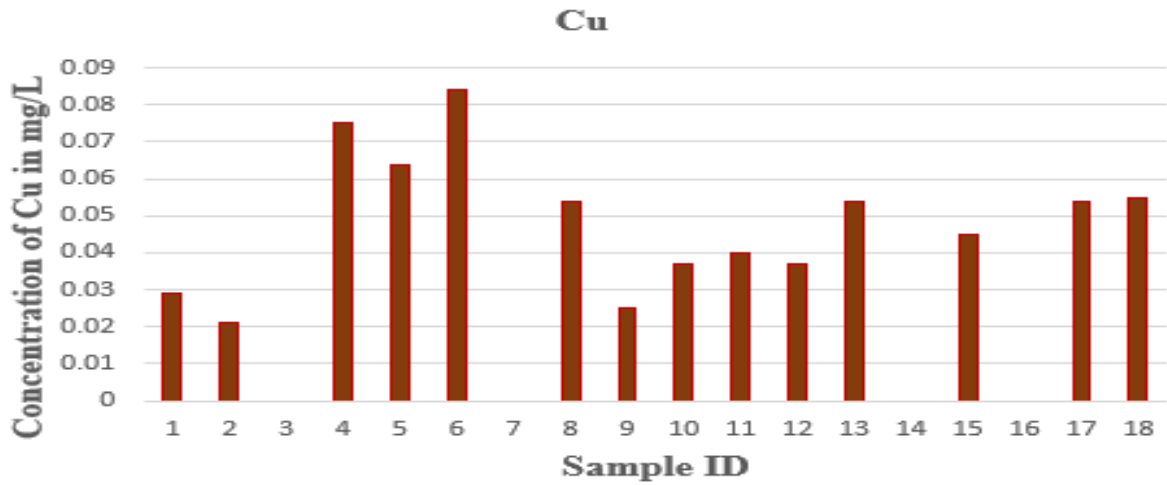


Figure 4.3: The average concentration of copper against Samples ID of the bottled water.

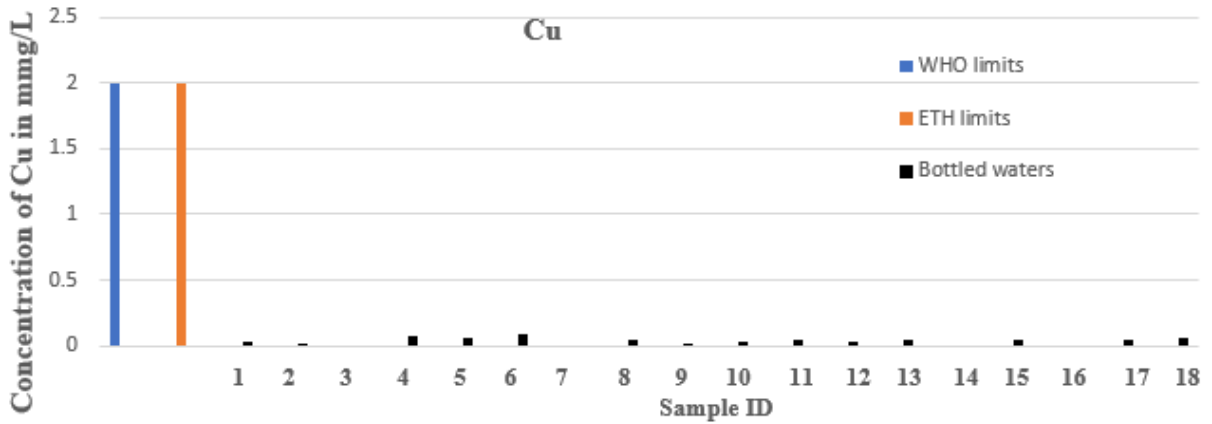


Figure 4.4: The average concentration of Copper (Cu) against samples ID and international standards.

4.1.1.3 The Concentration of Chromium (Cr) in the bottled water Samples

The result of the study showed that Chromium was detected in 12 bottled water samples. Its concentration varied from the lowest (0.044 ± 0.004) mg/L in Tseday bottled water sample to the maximum of (0.049 ± 0.002) mg/L in Sekt, Beza, Dangla, and south water sample as

presented in Figure 4.5 and Table 4.1. The comparisons of the values obtained from this study with national and international guide line is presented in Figure 4.6.

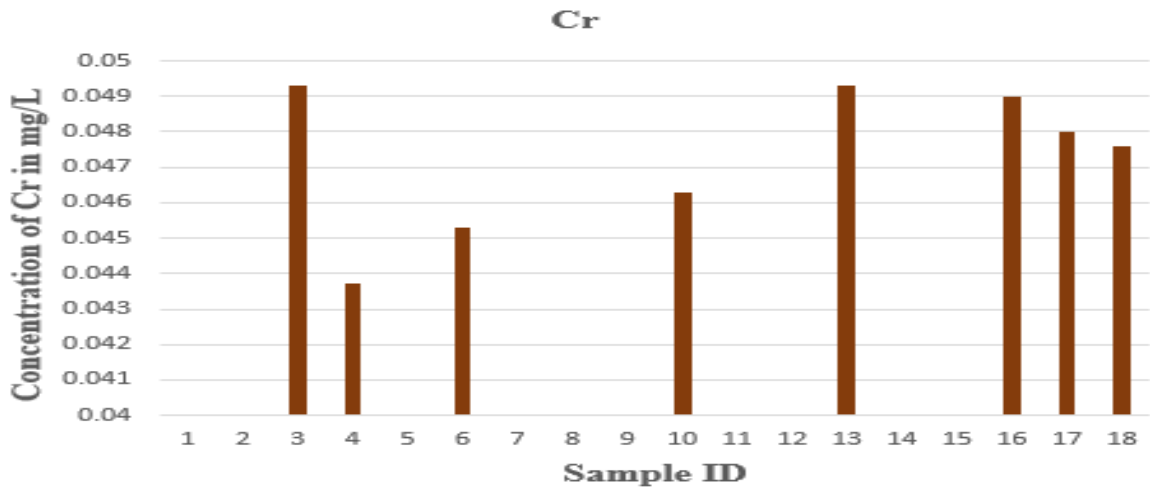


Figure 4.5: The average concentration of chromium against samples ID.

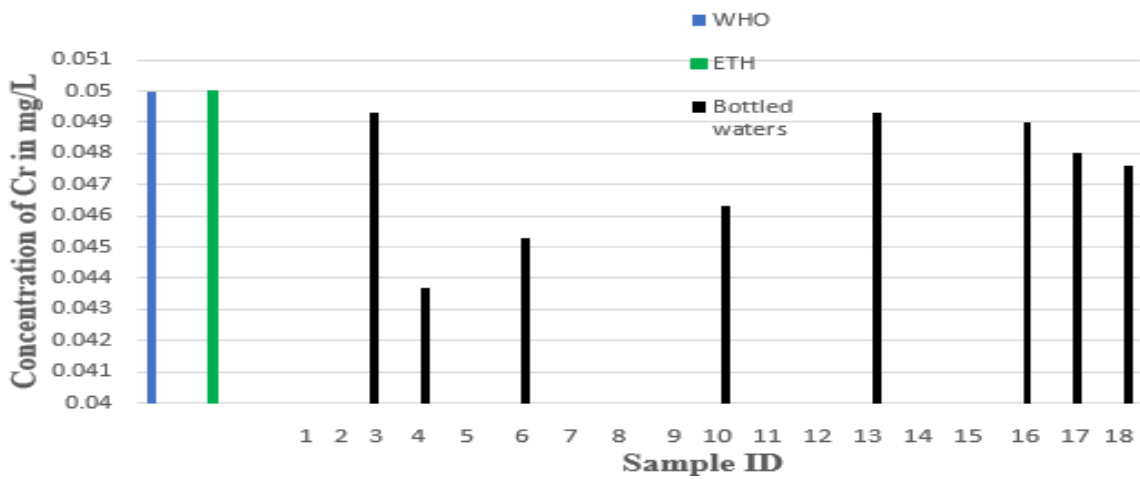


Figure 4.6: The average concentration of Chromium (Cr) against samples ID and comparison international standards.

4.1.1.4. The Concentration of Cobalt (Co) in bottled water Samples

Cobalt was not detected in all bottled water sample.

4.1.1.5 The Concentration of Lead (Pb) in bottled water Samples

Lead was detected in 18 bottled water samples. The result of the study showed that concentration of lead in bottled water varied from the lowest 0.005 ± 0.000 mg/L Beza water sample to the maximum of (0.0093 ± 0.0001) mg/L in Dear bottled water sample as presented in Figure 4.7 and Table 4.1. The comparisons of the values obtained from this study with national and international guide line is presented in Figure 4.8.

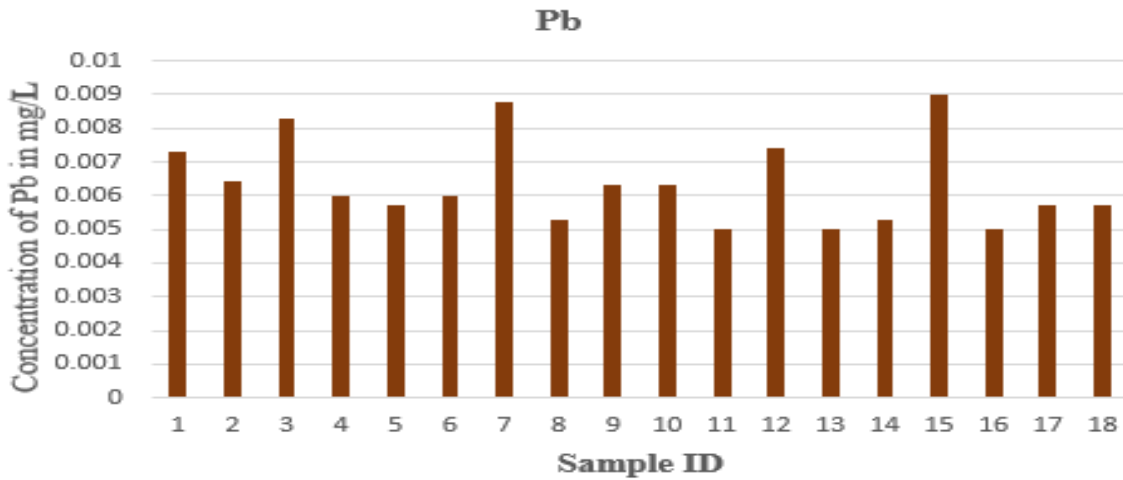


Figure 4.7: The avrage concentration of lead against samples ID of the bottled water.

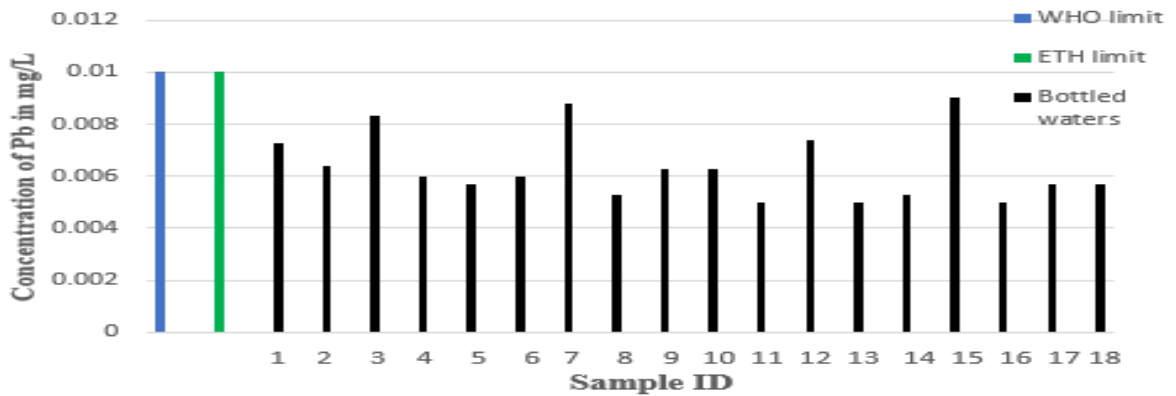


Figure 4.8: Comparison of the average concentration of Lead against international and standards.

4.2 Discussion

The discussion focuses on the detected heavy metals and their comparisons with similar studies globally.

Nickel (Ni): The concentration of Ni in samples ranged from (0.021 ± 0.001) mg/L in Choice bottled water sample to (0.068 ± 0.003) mg/L in Tseday bottled water sample (see Figure 4.1 and Table 4.1). Compared with other studies (Table 4.3) these results are smaller than a study by Olowoyo et al., (2022) in pritorya, South Africa, which reported a value of 0.075mg/L to 0.089mg/L, but are higher than those found by Sullivan (2011) in California, UAS a value of $(0.00024 - 0.00089)$ mg/L .

Of the 18 bottled water samples analyzed, 12 contained detectable levels of nickel but below the maximum permissible limit of 0.07mg/L (WHO, 2017) (see Fig.4.2). This indicates no potential health risk associated with nickel in these brands.

Coper (Cu): Copper concentration ranged from (0.021 ± 0.001) mg/L in Choice bottled water sample to 0.084 ± 0.004 mg/L in Gold bottled water sample (see Fig 4.3 and table 4.1). These findings are lower than those reported by Olowoyo et al.(2022) in pretoriya, Suth Africa where a concentration of 0.0407mg/L was recorded as indicated in Table 4.3.

Copper was bellowing detection limits in 5 bottled water samples, and the remaining 13 samples contained copper levels below the maximum permissible limit of 2 mg/L (WHO) (See Fig.4.4). Therefore, no health-related risks were associated with copper in all bottled water brands analyzed.

Chromium (Cr): Chromium concentration ranged from 0.044mg/L in Tseday water to 0.049 mg/L in Dangla water samples. Compared with other studies, Higher values have been reported in studies by Olowoye et al. (2022) in South Africa (0.0142 - 0.190)mg/L and by Bamuwamyie et al. (2017) in Kampala, Uganda (BDL - 0.107)mg/L.

Chromium was detected in bottled waters samples with values bellow the maximum permissible limit of 0.05 mg/L (WHO, 2024).

Cobalt (Co): Cobalt was not detected in all bottled water sample. Therefore, there is no health associated risk with it.

Lead (Pb): In our analysis, lead was detected in 18 bottled water brands with concentration (0.005 -0.0093) mg/L bellow the WHO permissible limit of 0.01mg/L (see Fig.4.8). This indicates no potential health risk associated with lead in these brands. In this study, the highest value found in Dear bottled water (0.0093 mg/L). Its source is ground water therefore the contamination may be associated due to urban prolusion since it is located Nefas silk, Addis Ababa. If this condition proceeds for a long period time the concentration may exceed the permissible limit. So that this signals strict quality control measure to be taken by concerned authorities.

Higher concentrations have been reported in studies conducted in Nigeria (0.348 mg/L by Mawell et al.,2018), Uganda (0.241mg/L by Bamuwamyie et al.,2017), and also in Suth Africa (0.123 mg/L by Olowoyo et al., 2022).

Finally, Comparision of the present study with different reports round the worled on heavy metals in bottled water is presented in Table 4.3 as shown bellow. From the table, one can see that the concentration of the metals analyzed in this study are, in most caese, in the rang of

different reported values though results of this study are close to the lower margins of the range. Higher values are observed in the literature probably due to different instrumentation, sample preparation, manufacturing process, or some difference in source area.

Table 4.3: Comparison of present study with other studies round the world on heavy metals in bottled drinking water.

Heavy metals	This study (mg/L)	Reported(literature) (mg/L)	References
Pb	0.005-0.0093	0.006 - 0.123	South Africa (Olowoyo et al., 2022)
		0.023 - 0.348	Nigeria (Maxwell et al. 2018)
		0.010 - 0.050	Uganda (Bamuwamy et al., 2017)
		0.091 - 0.241	India (Singla et al.2017)
		0 – 0.058	Iraqi (Jabbar et al. 2015).
		BDL - 0.00026	California (Sullivan,M.J., 2011)
		0.0001 - 0.0178	Canada (Pip, E., 2000)
Cr	0.044 -0.049	0.142 - 0.190	South Africa (Olowoyo et al., 2022)
		X	Nigeria (Maxwell et al. 2018)
		X	Uganda (Bamuwamy et al., 2017)
		BDL - 0.107	India (Singla et al.2017)
		0.000 - 0.016	Iraqi (Jabbar et al. 2015).
		0.0053 - 0.0143	California (Sullivan,M.J., 2011)
		X	Canada (Pip, E., 2000)
Ni	0.021 -0.068	0.075 - 0.089	South Africa (Olowoyo et al., 2022)
		0.003 - 0.0124	Nigeria (Maxwell et al. 2018)
		X	Uganda (Bamuwamy et al., 2017)
		BDL	India (Singla et al.2017)
		X	Iraqi (Jabbar et al. 2015).
		0.0024-0.0089	California (Sullivan,M.J., 2011)
		X	Canada (Pip, E., 2000)
Cu	0.021-0.084	0.021 – 0.407	South Africa (Olowoyo et al., 2022)
		X	Nigeria (Maxwell et al. 2018)
		0.020 - 0.120	Uganda (Bamuwamy et al., 2017)
		0.034 - 0.098	India (Singla et al.2017)
		0.034 - 0.098	Iraqi (Jabbar et al. 2015).

		0.001-0.006	California (Sullivan,M.J., 2011)
		0.0001-0.0165	Canada (Pip, E., 2000)
Co	ND	0.0001-0.001	California (Sullivan,M.J., 2011)

X: not assessed by the author; BDL: below detection limits

In general, the result of the study indicates varying levels of heavy metal concentration across different brands of bottled waters. In terms of safety none of the detected heavy metals exceeded the typical regulatory limits for drinking water. But our findings highlight the need for further investigation on bottled water by using more sensitive spectroscopic techniques to produce more reliable results for formal authorities' decisions.

4.3 Method Validation and Quality Assurance

This section discusses the validation of FAAS method. The parameters that were used include, linearity test, detection limits, precision of measured data, and recovery test.

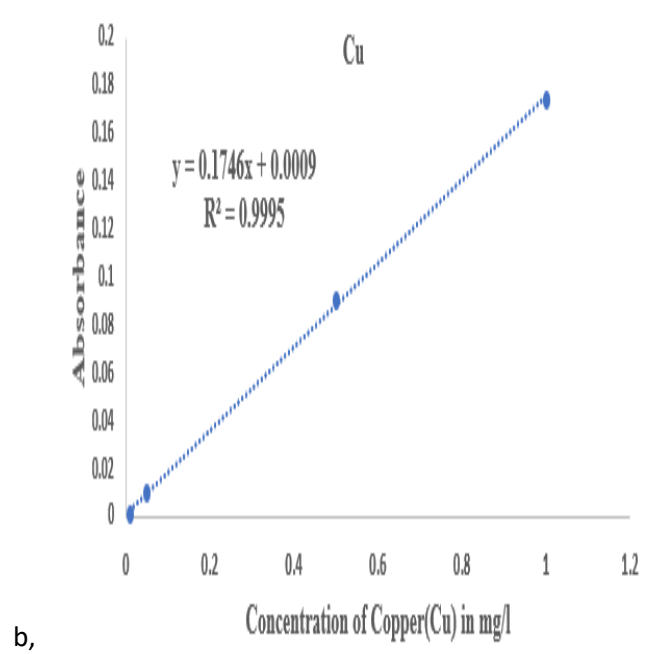
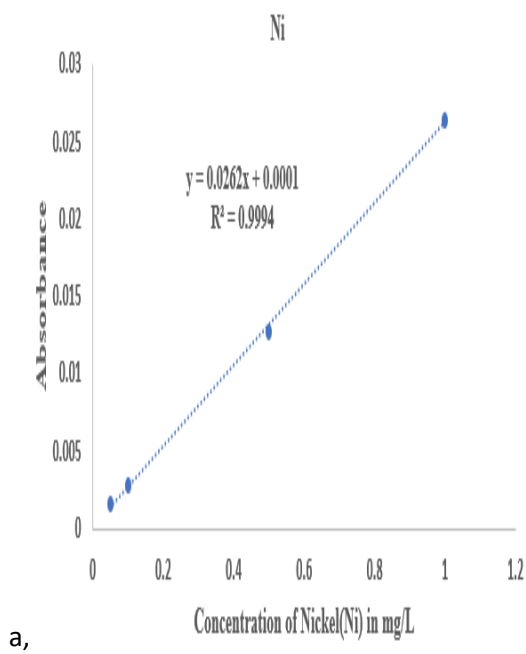
4.3.1 Calibration and Linearity of Instrumental Response

The calibration of the instrument was done by running a series of different concentrations of standard solutions prepared separately for each heavy metal. Hence, calibration curve showing absorbance versus concentration at specific wavelength were prepared for each heavy metal through direct analysis of the instrumental blank and five calibration metal standard solutions as shown in Table 4.4. And the calibration graphs for the five heavy metals (Pb, Cr, Ni, Cu and Co) were obtained by using suitable standard solutions prepared from stock solutions are given in Figure 4.9(a-e) shown below.

Table 4.4: Concentration in (mg/L) and absorbance in absorbance unit data for Cr, Ni, Cu, Co, and Pb.

Cr		Ni		Cu		Co		Pb	
Cnc.	Abs.	Cnc	Abs.	Cnc.	Abs.	Cnc.	Abs.	Cnc.	Abs.
0.05	0.004285	0.05	0.001552	0.01	0.001547	0.05	0.00138	0.05	0.001268
0.5	0.03792	0.1	0.002797	0.05	0.009521	0.5	0.023458	0.1	0.002618
1	0.075514	0.5	0.012798	0.5	0.090724	1	0.043329	0.5	0.014801
2	0.1541	1	0.02648	1	0.174248	2	0.086107	1	0.030247

Cnc. - concentration, Abs.- absorbance.



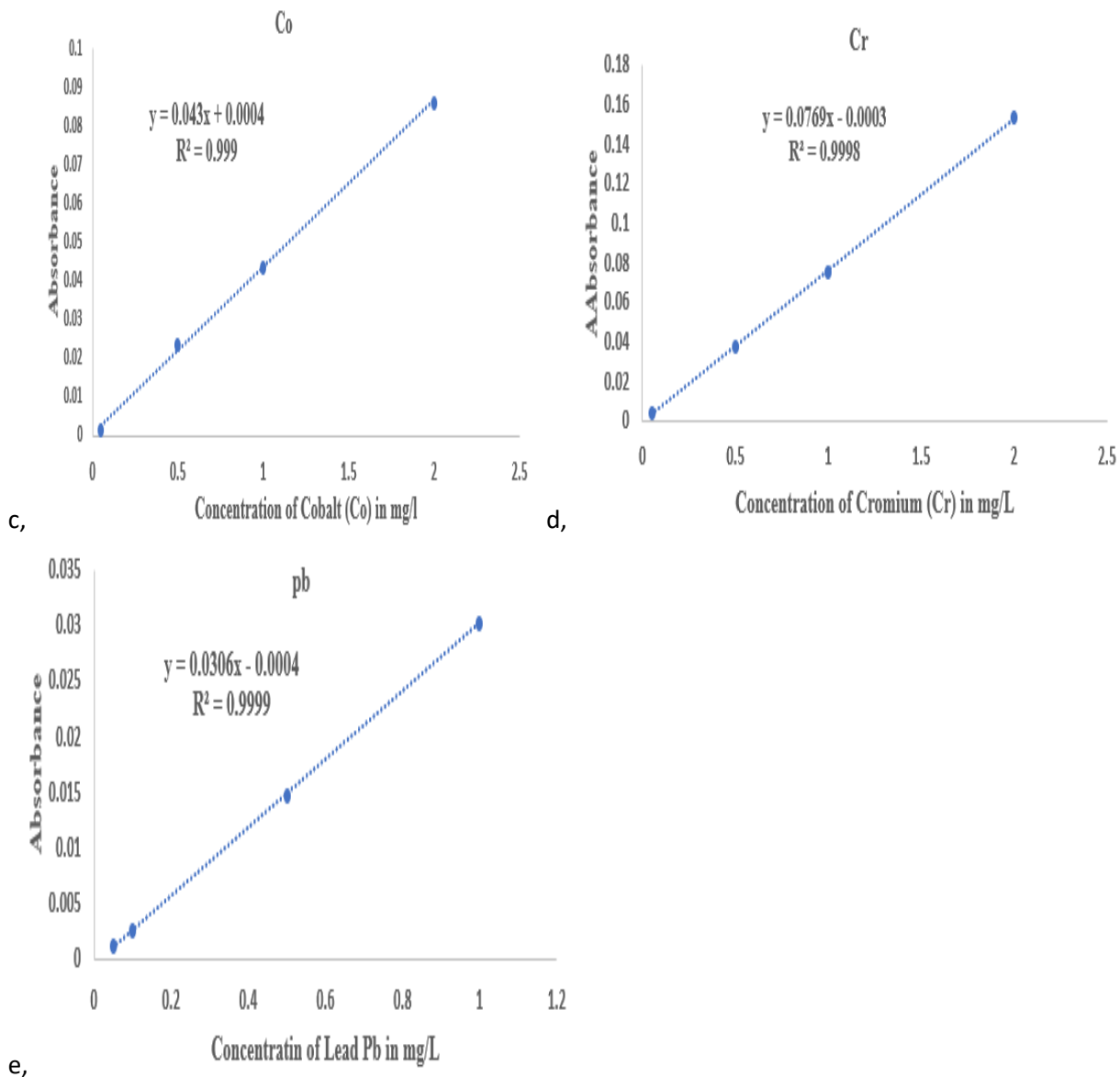


Figure 4.9(a-e): Standard calibration curve of the selected heavy metals.

As observed from the calibration curves the correlation coefficient values for Pb, Cr, Ni, Cu and Co are 0.9999, 0.9998, 0.9994, 0.9995, and 0.9990 respectively this is indicated in table (Table 4.1) with their regression equation. The values are greater than the minimum linear correlation coefficient 0.999 set by (Miller et al., 2010). Therefore, there is a linear relationship between instrument response(absorbance) and standard concentration of each

heavy metal indicating that the method is accurate and can be employed for the determination of unknown concentration in the respective samples immediately after calibration.

4.3.2 Detection Limits

The calculated instrumental detection limit by using equation 3.1 for each heavy metal by the implemented analytical methods are given in table (Table 4.4). The values are for Cr (0.040 mg/L), Ni (0.032 mg/L), Cu (0.023 mg/L), Co (0.070 mg/L), and Pb (0.014mg/L). This shows the results are comparable with the ideal instrumental detection limits (IDL).

Table 4.5: Determined regression equations with its detection limits.

Heavy metals	Linear regression equations	Correlation Coefficients (R ²)	Ideal Instrument Detection limits (IDL)	Experimental value of Detections limits (DL)
Cr	Y= 0.0769X - 0.0003	0.9998	0.040	0.040
Ni	Y=0.0262X + 0.0001	0.9994	0.020	0.032
Cu	Y=0.1746X + 0.0009	0.9995	0.005	0.023
Co	Y=0.0430X + 0.0004	0.9990	0.050	0.070
Pb	Y= 0.0306X - 0.0004	0.9999	0.004	0.014

The calibration equation was used to show the relation between an independent variable x (concentration) and a dependent variable Y (absorbance signal). In regression the line has form of $Y = mx + b$, where y is dependent variable that is absorbance signal; x an independent variable (concentration), m slope of the line which tells us how much the absorbance signal changes for a corresponding change in concentration, and b the y- intercept.

4.3.3 Precision of the Measured Result

The precision of the analytical method was evaluated as percent relative standard deviation (%RSD) using equation 3.2. The precisions of the measured heavy metals have lied in the range of 0.21% - 8.5% for Ni; 0.0% - 8.8% for Cu; 0.2% - 9% for Cr; and 0.0% - 13.04% for Pb. Based on Gonzalez et al., (2007), the highest relative standard deviation values acceptable for concentration of less than or equal to 1 ppm (1mg/L) is 16%. Hence the result showed reasonable repeatability accuracy because precisions are in the acceptable range.

4.3.4 Accuracy

The accuracy of the method evaluated as recovery tests using equation 3.3. It was performed by adding a known concentration, round a mid-value, of heavy metals solution on the bottled water samples. The results are presented in table (Table 4.5). The mean recoveries for Pb, Ni, Cr, and Cu were 92.6%, 101.7%, 100.7%, and 98.4% respectively

Table 4.6: Average recovery for Pb, Ni, Cr, Co, and Cu.

	Pb	Ni	Cr	Cu
Concentration in control sample(mg/L)	0.006±0.0006	0.045±0.002	0.034±0.003	0.054±0.003
Added amount(mg/L)	0.05	0.05	1	0.05
Measured concentration	0.052 ± 0.001	1.062 ± 0.003	1.041±0.002	0.103±0.001
Sample				
Recovery	0.046	1.017	1.007	0.049
% Recovery	92.6%	101.7%	100.7%	98.4%

In summary, the methods validation demonstrated that FAAS technique employed in this study is in the acceptable region for the determination of heavy metals (Pb, Cr, Ni, Cu, and Co) in bottled water samples. The calibration curves showed acceptable linearity, the detection limits were suitable for the levels of metals expected in the samples, and the recovery studies confirmed the accuracy of the method. The precision, as indicated by the low %RSD values, further validates the reliability of the method.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research we have used flame atomic absorption spectroscopy (FAAS) for the detection and determination of heavy metals in drinking bottled water samples. This was performed by measuring the absorbed light intensity obtained from light- matter interaction. The findings of the study indicated the presence of some heavy metals in the bottled waters samples though it is below the permissible limits.

We measured the concentration of selected heavy metals (Pb, Ni, Cr, Cu, and Co) in 18 different brands of bottled water that were collected by clustered random sampling techniques from markets of Ethiopia considering different geographic locations: East and west Amhara region; round Addis Abeba; central Oromiya, West oromiya, south Oromiya, North Oromiya; Meakelawi Ethiopia region; and Sidama region.

For heavy metal analysis the laboratory sample preparation was performed by acid treatment. Nitric acid was added to the sample to acidify and to preserve the metals from precipitation and adsorption.

Before the measurement process started FAAS was calibrated to ensure accurate and reliable quantitative analysis. For the calibration process a series of standard solutions containing known concentration of the element of interest was prepared by diluting a stock solution (1000mg/l) for each heavy metal. During the calibration process the instrumental parameters

(most sensitive elemental wave length, lamp current, slit width and flame conditions) were optimized to enhance maximum sensitivity and reduce interference.

The detected concentrations for Ni, Cu, Cr, Co, and Pb were found to be in range of (0.021 - 0.068) mg/L, (0.021 - 0.084) mg/L, (0.044 - 0.049) mg/L, (0.000) mg/L, and (0.005 – 0.009) mg/L respectively.

The validation of the analyzed data was assessed by linearity of the instrumental calibration as correlation coefficient with value $R^2 > 0.9990$ which is in the acceptable region as stated by Gonzalez and Herrador (2007), precision of the measured data for all metals has lied in the range of 0.00% -13.04 % for Pb, 0.2% - 9% for Cr, 0.21% - 8.5% for Ni, and 0.00% - 8.8% for Cu. Where all values have lied bellow the maximum value of 16% stated by Gonzalez & Herrador (2007). And recovery test has lied in the range of 92.6% to 101.7% which is in the acceptable range of 90% - 110% as stated by Gonzalez, & Herrador, (2007). Where all operating within the acceptable guideline.

The current study results ware compared with Ethiopia's (2002) and WHO's (2004) international guide lines for drinking water quality. All the heavy metals Copper, Cobalt, nickel, chromium, and lead ware below the established guide line in all 18 samples of bottled water brands. Due to the limitation of literature review on bottled spring water in the country the results were not compared. Generally, the findings of this study serve as a starting point for further study.

5.2 Recommendation

More advanced and sensitive spectroscopic techniques have come in to work for environmental control in heavy metal pollution. To produce reliable information on quality of bottled water as per regulatory requirements, we recommend that:

-Bottling companies should be enforced by law to deliver full information concerning heavy metals on product labeling or packaging material of bottled water so that we can compare analyzed values with labeled values.

-Regular supporting, monitoring, testing, and licensing for heavy metals content of bottled waters should be conducted by concerned authorities.

-Further studies should be conducted to assess the heavy metals contamination by using most sensitive and advanced spectroscopic methods for the sake of comparison of the results of this study and to produce more reliable information.

-Also, correlational studies can be conducted between heavy metals content of the source area and the bottled natural spring water.

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