ADDIS ABABA UNIVERSTY

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DEPARTEMENT OF CHEMISTRY

Determination of Selected Heavy Metals in the Leaf of Vernonia Amygdalina Collected From Gurage Zone Sodo Woreda Using Atomic Absorption Spectroscopy Technique

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By: Belay Belete

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SCIENCE

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September 2024

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DECLARATION

I declare that the thesis entitled Determination of heavy metals in the leaf of vernonia amygdalina (bitter leaves) collected from Gurage zone Sodo woreda using Atomic Absorption Spectroscopy Technique is my original work and has not been submitted for the degree in any other universities.

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ABSTRACT

This study aimed to determine the concentration of heavy metals (Cd, Ni, Cr, Cu, and Pb) in leaf of *vernonia amygdalina* in Amharic 'Grawa' using flame atomic absorption spectrometry. An optimized digestion procedure was selected based up on less reagent consumption, digestion time and temperature. 0.5 gram of oven dried sample was digested by mixing 3 ml of 69% HNO_3 and 1 ml of 70% $HClO_4$ at a temperature of 270℃ for 2:30 hour due to minimum time, temperature and reagent conception. The leaf of heavy metals (Cr, Cd Cu, Pb, and Ni) in vernonia amygdalina was determined and the obtained concentrations of these metals were compared with the limit values set by WHO. The mean concentrations of the selected heavy metals were determined from triplicate samples of *vernonia amygdalina*, which were grown in different areas of Sodo Buee. In this study the mean concentrations of heavy metals obtained in *vernonia amygdalina* samples in mg/Kg were; Pb (0.3944±0.0096), Cu (0.18867±0.00236), Ni (0.0429±0.023), Cd (0.0191±0.028), and Cr (0.0150±0.00086). The mean concentration of heavy metals in vernonia amygdalina were in the order of Pb > Cu > Ni ≻ Cd >Cr. The concentration of all analyzed metal in vernonia amygdalina sample was below the maximum allowable concentration for *V.amygdalina* leaf given by WHO except lead which is comparable to WHO permissible limit this was due to different value in different year. These suggest to they were safe in terms of health risk perspective for used as medicine plant. Low RSD values, as seen in for Pb and Cu, suggest that the FAAS method was effective for these metals. The concentrations metals in this specific area are comparable to values for plants reported in other parts of the world. However, higher RSD values, for Ni, indicate potential challenges in measurement precision, possibly due to low concentrations or other experimental factors. The validity of the optimized procedure was evaluated by the analysis of spiked samples whose recoveries were in the range 92-98%.

Key words: vernonia amygdalina, heavy metals, FAAS.

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List of Abbreviations

ESADDI	Estimated safe and adequate daily intake
AAS	Atomic Absorption Spectrometer
FAAS	Flame Atomic Absorption Spectrometry
CSAAS	continuous Source Atomic Absorption Spectrometry
ETAAS	Electro thermal Atomic Absorption Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
ATSDR	Agency for Toxic Substance and Disease Registry
EDL	Electrode-less discharged lamp
EPA	Environmental Protection Agency
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
HDL	High Density Lipoprotein
HCL	Hollow Cathode Lamp
ICP-OES	Inductively-Coupled Plasma Optical Emission Spectroscopy
LSAAS	Line Source Atomic Absorption Spectrometry
MDL	Method Detection Limit
OSHA	Occupational Safety and Health Administration
RDA	Recommended Dietary Allowance
RO	Reverse Osmosis
ROS	Reactive Oxygen Species
RSD	Relative Standard Deviation
WHO	World Health Organization
FAO	Food and Agricultural organization

1. INTRODUCTION

1.1. BACK GROUND OF THE STUDY

Vernonia amygdalina is a shrub that grows throughout Africa and South-Asia and belongs to the family Asteraceace, Kingdom Plantae and species vernonia amygdalina. It is commonly called bitter leaves because of its bitter taste and is used as vegetables in soups. It is one of such leafy vegetables which are known for its enormous medicinal and health potentials.

Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons and them ranking amongst the major contaminants of leafy vegetables [2, 3]. These leaf vegetables are very essential protective food and are useful for the maintenance of health, prevention and treatment of diseases [4]. *Vernonia amygdalina* is one of such leafy vegetables which are known for its enormous medicinal and health potentials. It is commonly called bitter leaf because of its bitter taste and is used as vegetables in soups. The bitter taste of *V. amygdalina* has been attributed to its anti-nutritional components such as alkaloids, saponins, glycosides and tannins [5]. it is also referred to by several local name in different language of different regions In Ethiopia, bitter leaf is known as" grawa' in Amharic .and ebicha (oromo).

Figure 1: bitter leaf (Vernonia amygdalina)

V. amygdalina has been widely used for the traditional treatment and/or management of various diseases in human and animals in Africa [8]. *V.amygdalina* has been reported to contain a number of phytochemicals including saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignans, xanthones, anthraguinones, edotides and sesquiterpenes Also V. amygdalina has been shown to possess significant amount of proteins, Carbohydrates, fiber, calcium, iron, potassium, phosphorus, manganese, copper and cobalt [6]. Furthermore, V. amygdalina has been reported to possess antifungal [7], anti-malarial [8], anticancer, antioxidant [9], anti-diabetic [10], analgesic activity [8], and anti-inflammatory properties [11]. However, despite all these numerous health benefits of V. amygdalina, it tends to bio accumulate heavy metals and intake of heavy metal contaminated vegetables pose a risk to human health and well-being. More so, a number of studies have shown heavy metals as important contaminants of vegetables [12].

In Ethiopia the plant is used in cleaning the containers used for fermentation purpose, Due to its bitterness, it also can be used as a bittering agent, a hop substitute and for the control of microbial contamination in beer brewing without affecting the quality of malt and also it is used to make honey wine called" Tej" The leaves are used for human consumption and washed before eating to get rid of the bitter taste. They are used as vegetable and stimulate the digestive system [13].

The content of essential elements in plants is soil being affected by the characteristics of the soil and the ability of plants to selectively accumulate some metals

Additional sources of heavy metals for plants are: rainfall in atmospheric polluted areas, traffic density, use oil or treatment to plant to complete the Maturation of some crops or to give crops attractive look to consumer.

Removal of heavy metals from contaminated samples has been performed by a variety of approaches. Examples for these techniques include ion-exchange, reverse osmosis (RO), chemical precipitation, hydride generation, electrolysis, cold vapor atomic absorption and sorption. Yet, most of these techniques are time, cost, chemical and energy consuming. Moreover, most of these procedures are not efficient for heavy metals traces.

Many different instrumental methods have been used to determine the metal concentration of foods (fruits). These methods which on spectroscopic studies of atoms or of elementary ions with ultraviolet and visible radiation; can be performed only in a gaseous medium in which the

individual atoms or ions are well separated from one another. Some of the commonly used methods for the determination of the concentration of heavy metals in a given sample are: The Atomic Absorption Spectrometry; includes: Flame Atomic Absorption Spectrometry, Graphite Furnace Atomic Absorption Spectroscopy, Inductively Coupled Plasma-Mass Spectrometry and Inductively Coupled Plasma-Absorption Emission Spectrometry and Inductively Coupled Plasma-Optical Emission Spectrometry. From those Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environment samples [14]**.** Therefore, this study investigated some selected heavy metal contents of *vernonia amygdalina* leaf gathered from Gurage zone Sodo woreda in central Ethiopia region by using flame atomic absorption spectroscopic method (FAAS).

1.2. Statement of the problem

Vernonia amygdalina (bitter leaf) is very important to cure a number of diseases and hence used by most of individuals in the country in a regular basis. It is also known that the source of mineral nutrients for human being is plant materials consumed in the form of food or medicine. Thus, it is very important to assess the essential, toxic mineral nutrients that can be accumulated in the stated plant species so as to address the individual daily intake of mineral nutrients. Furthermore, dosages of traditional medicines are not precisely understood. So, individuals may take these traditional medicines in larger quantity. Therefore, beside toxicity from the active ingredients, the individuals may suffer from heavy metal toxicity and hence their normal body function will be affected as toxic metals are responsible for most of our body enzymatic activity. Therefore, determination of toxic heavy metals in the plant extract is very important to ensure individuals health status. Furthermore, the result of this study may help to propose the maximum dosage of the plant for normal body function in terms of heavy metal content. Based on this finding the local expertise will try to manage the normal dosage by integrating their experience with the one we are going to report optimum quantity. Since *vernonia amygdalina (bitter leaf)* is one of the main traditional medicines and serve to cure different diseases for human being and domestic animals, the knowledge of their mineral concentrations are of particular interest. However, information on the contents of heavy metal elements in the different parts of the plant extract is scares in the literature. Heavy metals presence such as Cd, Cr, Cu, Mn, Ni, Pb and Zn in food is one of the most harmful health problems in the world due to its non-biodegradable nature, persistence and toxicity. People are exposed to heavy metals

and metalloids through ingestion, inhalation, and dermal contact. Therefore, contamination of the food chain is one of the important pathways for the entry of these contaminants in the human body which can cause health risks. Due to those problems I initiate to study the concentration of heavy metals in this herbal medicine.

1.3. Objectives

1.3.1. General objective

- To determine the concentration of selected heavy metals (Cd, Ni, Cr, Cu, and Pb) in *vernonia amygdalina* (*bitter* leaf).

1.3.2. Specific objectives

- To optimize the process parameters (time, temperature and reagents) for digestion of *vernonia amygdalina* (bitter leaf)
- To compare the concentration of selected heavy metals in *vernonia amygdalina* with those set in guide lines
- To determine the level of selected heavy metal (Cd, Ni, Cr, Pb, and Cu) in *vernonia amygdalina* using FAAS.

1.4. Significance of the Study

This study provides important information on the levels of selected heavy metals grown in the central Ethiopia regional state in Sodo Buee town that the society could be free of the potential health risks caused from the too much uptake of the heavy metals in the herbal medicinal plant. On the other hand, the results of this study could be used as reference for other researchers who want to conduct similar studies on the same plant growing in different parts of the country Furthermore, the findings of this study will provide adequate information on the distribution of heavy metals in the leaves. To estimate the reliability of the test experiments of the samples measurements were conducted in triplicate and the mean of the result was taken.

2. LITERATURE REVIEW

2.1. Heavy Metals

Heavy metals are generally referred to as those metals which possess a specific density of more than $5g/cm^3$ and adversely affect the environment and living organisms Heavy metals are among the most serious environmental contaminants which are coming from different sources. Soil, as one of the main components of the environment, is the site of heavy metal entry into plants and consequently into the food chain [1]. Herbal medicinal plant *vernonia amygdalina* contain a significant quantity of heavy metals of great importance is the fact the heavy metals, otherwise called toxic metals such as lead, cadmium, mercury, manganese, arsenic, are detrimental to human health for a verity of reasons and unfortunately are found prevalent in the environment as a result of the activities of man in the modern society. Heavy metals are significant environmental pollutants and their toxicity is a problem of enhancing significance for ecological, evolutionary, nutritional and environmental reasons [2]and they ranking amongst the major contaminants of leaf vegetables are very essential protective food and are useful for the maintenance of health prevention and treatment diseases [4].Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered as toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer [15].

2.2. Selected heavy metals

2.2.1. Copper

Copper is the earliest metal human used. The average abundance of copper in the earth crust is 55mg/kg. In nature, copper mainly exists in the form of sulfide and oxide ores and its distribution is very wide. The content of normal copper in soil is 2-200mg/kg and the average is 22mg/kg **[16]**. The average daily intake of copper in the US is about 1 mg Cu with the primary source being the diet. The bioavailability of copper from the diet is about 65-70% depending on a variety of factors including chemical form, interaction with other metals, and dietary components. The serum copper concentration ranges up to approximately 1.5 mg/L in healthy persons. Gastrointestinal symptoms occur at whole blood concentrations near 3 mg Cu/L **[17].** Copper is critical for energy production in the cells. It is also involved in nerve conduction,
Connective tissue, the cardiovascular system and the immune system. Copper is closely related to estrogen metabolism, and is required for women's fertility and to maintain pregnancy. Normal Values of Cu in Serum = 12 - 26 µmol/L and Urine = 0.05 - 0.55 µmol/day Deficiency of copper effect upon thyroid function caused Vascular Lesions Central nervous system disorder and convulsion, Hair abnormalities [18] hyper-Copper caused Decreased hemoglobin and erythrocyte levels, Death and Cancer [19].
Copper is an essential element for plant nutrients, but it becomes toxic at high concentrations. Copper is required for constituting enzymes catalyzing redox reactions, and is involved in photosynthetic functions **[20]**. Excess copper induces high levels of reactive oxygen species (ROS) and affects the photosystem in photosynthesis **[21]**, which subsequently reduces the yield or quality of crops. The toxic effects of copper are studied in several crops. When the soil's copper level is over 300 mg/kg, the rice grain yields a decrease of about 50% **[22**]. Excessive intake of Cu can cause irritation of the upper respiratory tract, abdominal pain, diarrhea, vomiting, and liver damage [60]

2.2.2. Chromium

Chromium exists in a series of oxidation states from -2 to +6 valence. The most important stable states are 0 (elemental metal), +3 (trivalent), and +6 (hexavalent). Chromium in chromite ore is in the trivalent state; industrial processes also produce the elemental metal and hexavalent chromium. The health effects of chromium are primarily related to the valence state of the metal at the time of exposure. Trivalent (Cr [III]) and hexavalent (Cr [VI]) compounds are thought to be the most biologically significant. Cr (III) is an essential dietary mineral in low doses. Cr (VI) compounds are carcinogenic. Cr (VI) is generally considered 1,000 times more toxic than Cr (III) [23]. Essential Dietary Nutrient Cr (III) is an essential dietary nutrient. It is required to potentiate insulin and for normal glucose metabolism. Important for insulin and improve in hypoglycemic patients following chromium supplementation. Another effect of chromium supplementation that could be a result of its potentiation of insulin sensitivity is the redistribution of body fat, protein and water [24].

Estimated Safe and Adequate Daily Dietary Intake (ESADDI) bar day about 10-200µg/day different age lower this dose caused problem in insulin system and over dose caused Weakened immune systems, Kidney and liver damage, Alteration of genetic material, Lung cancer and Death [25].Cr(III) deficiency has been associated with cardiovascular disease, decreased lean body mass, decreased sperm count, elevated percent body fat, fasting hyperglycemia, glycosuria, impaired fertility, impaired glucose tolerance, and maturity-onset diabetes.

Cr (III) is found in most fresh foods and drinking water. Dietary sources rich in Cr (III) include: breads, cereals, fish, fresh vegetables, meats, and spices. Other significant sources of Cr (III) are mineral supplements, brewer's yeast, and beer. The National Academy of Sciences has established a safe and adequate daily intake for Cr (III) in adults of 50 -200 micrograms per day. On the average, adults in the United States take in an estimated 60-80 micrograms of Cr (III) per day in food. Therefore, many people's diets may not provide enough Cr (III) [26]. The biologically active form of an organic Cr (III) complex, often referred to as glucose tolerance factor (GTF), is believed to function by facilitating the interaction of insulin with its cellular receptor sites. Studies have shown that the Cr (III) supplementation in deficient and marginally

deficient subjects can result in the rapid reversal of many of the symptoms of chromium-deficiency [26, 27].

2.2.3. Cadmium

Cadmium is a soft, malleable, bluish white metal in zinc ores and to a much lesser extent in the cadmium mineral greenockite. Most of the cadmium produced today is obtained from zinc by products and recovered from spent nickel-cadmium batteries. Only a small amount of cadmium remains in the body after eating food contaminated with cadmium, but if consumed over a long period of time can lead to kidney disease and cause bone weaker large amounts of Cd can damage the kidney liver and heart and in severe cases may cause death. Cadmium occurs naturally in only trace concentrations in agricultural soils. Contamination of agricultural soils with Cd is derived from sources, such as phosphate fertilizers manufactured from rock phosphates high in Cd and by the application of sewage sludge to a greater extent and by the pesticides and gypsum to lesser extent. Zinc smelters in the vicinity of agricultural soils can also be significant contributors to soil contamination with Cd. Food crops grown on contaminated soils may take up substantial amounts of Cd and this could result in Cd entering the food chain of animals and humans when consumed [28]. There is now general concern that under certain conditions the Cd content of plants may be raised and thus become hazardous to man. The sources of soil Cd are varied. Cadmium is added to soils in very small amounts in phosphate fertilizers. Along with other heavy metals, it is also present in sewage sludge. Levels from about 10 to as much as 1500 ppm Cd have been observed in the dry matter of sewage sludge, which is being used more and more on agricultural land [29]. There is considerable current interest in Cd in plant nutrition. Normal Cd levels in plant material are in the range of 0.1 - 1.0 ppm. Although the roots of several species can take up large quantities of Cd from solution, the movement of Cd through the plant is restricted. Cadmium appears to be held in the roots on exchange sites, and can be replaced by Ca^{2+}, Mn^{2+}, and Zn^{2+}. As Ca^{2+} is normally the dominant cation in soil solution it may substantially affect the uptake of Cd from the roots to the tops is particularly depressed by phosphate [29]. Cadmium and Zn are chemically very similar. Cadmium is thus able to mimic the behavior of the essential element Zn in its uptake and metabolic functions. Unlike Zn, however, Cd is toxic both to plants and animals. The basic cause of toxicity

probably lies in the much higher affinity of Cd for thiol groupings (SH) in enzymes and other proteins. The presence of Cd therefore disturbs enzyme activity [28]. Most recently, interest in Cd has been directed at progressive accumulation in biological systems at low levels at which Cd generally occurs environmentally [30]. Toxic effects in man have been observed from the regular consumption of plants in excess of 3 ppm [28]. Continued exposure to small amounts of Cd leads to accumulation, in human and animal liver and kidney tissues resulting in damage and malfunction of these organs [29]. It disturbs the metabolism of Ca and P and cause bone disease, which is very painful, and causes excessive demineralization and embrittlement of the skeleton [27].

2.2.4. Lead

The total Pb content of agricultural soils lies between 2 - 200 ppm. Soils with levels in excess of this are limited to a relatively few regions where Pb mineral deposits occur. Lead airborne contamination in soils is usually restricted to the top few cm of the soil profile. This retention in the upper part of the soil profile probably relates the strong adsorption of Pb^{2+} to organic and clay colloids as well as to the formation of insoluble Pb chelates with organic matter. The availability of soil Pb is usually low. A high soil pH may precipitate Pb as hydroxide, phosphate, or carbonate as well as possibly promoting the formation of Pb organic matter complexes [31]. Lead is a major chemical pollutant of the environment, and is highly toxic to man. No other pollutant than Pb has accumulated in man to average levels so close to those which are potentially clinically poisonous. Lead is toxic because it mimics many aspects of the metabolic behavior of Ca, and inhibits many enzyme systems. In animals, Pb toxicity interferes with Fe metabolism and the formation of hemoglobin [31]. Lead reaches soil and plant cover as an aerial deposit and in precipitation, irrigation water, mine drainage, leaf litter, or ground dust blown in from elsewhere. Pb is also added to soil as pesticide, such as Pb arsenate, or as an impurity in certain fertilizers such as limestone and superphosphates. Two pathways are available for Pb to enter plants: uptake by the roots and uptake by the foliage. Once inside the system, Pb seems to be retained by cell membranes, mitochondria, and chloroplasts [32]. Lead enters man by inhalation and ingestion. Absorbed and carried by the blood, it is accumulated in liver, kidney, and bone up to about the fifth decade of life [32]. Pb causes brain damage

particularly to the young. There is evidence that Pb pollution can induce aggressive behavior in animals which can also occur in humans [31]. Pb is not essential elements that are required neither in the human body nor in plants, and which cause various bimolecular adverse functional effects at low level doses,

2.2.5. Nickel

Nickel is a metallic element with a silvery-white metal, shiny appearance .it is the fifth most common element on earth and occur extensively in the earth crust and core. Nickel along with iron, is also a common element in meteorites .it occurs naturally in soil and water.it is also an essential nutrient for plants. It is used to make coins, wires, in gas turbines and rocket engines as it has the capability to resist corrosion even at high temperature. Accumulation of nickel and its compounds in a body through chronic exposure may be responsible for a variety of adverse effects on the health of human beings, such as lung fibrosis, kidney and cardiovascular diseases and cancer of the respiratory tract [33]. nickel has very valuable properties there for this element is mostly used as an ingredient of steel and certain alloys ,also in production of catalyst ,batteries and in the electrical engineering industry its product nickel based catalyst have an important role in the reaction between organic compounds .however ,nickel is well known as a toxic metal. The releasing of nickel in the environment it pollutes the environment plants and animals. However, nickel control in different object is performed by physical and it can determine by AAS method [34].

2.3. Atomic Absorption Spectrophotometry

Atomic absorption spectrometry (AAS) is one of the most often used techniques for the quantitative determination of elements in environmental materials at trace and ultra-trace levels. This is done by reading the spectra produced when the sample is excited by radiation. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample. A detector measures the wavelengths of light transmitted by the sample and compares them to the wavelengths which originally passed through the sample [34]. A signal processor then integrates the changes in wavelength absorbed which appear in the

readout as peaks of energy absorption at discrete wavelengths. Every atom has its own distinct pattern of wavelength at which it will receive energy, due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample **[35].**

AAS is an optical atomic spectrometric technique based on the measurement of the specific absorption originating from free non-ionized atoms in the gas phase. To transfer the analyte to free atoms, different types of atomizer are in use, the flame and the graphite furnace types being the most often used. Typical detection limits of flame atomic absorption spectrometry (FAAS) are of the order of 1-100 ppb, making it a perfect tool for the determination of minor and trace elements, at least for contaminated samples. Graphite furnace atomic absorption spectrometry (GFAAS), offering detection limits which are about a factor of 20–200 times lower than for FAAS, is the standard method for many trace elements, especially for background values, and for unpolluted samples, such as fresh water and biological materials. AAS in its conventional configuration is a single-element technique, which has to be used in a sequential mode when more than one element has to be determined. However, there are commercial instruments available that can be used for the determination of 6–8 elements simultaneously **[36].**

Atomic absorption spectrometry has many uses in different areas of chemistry such as clinical analysis of metals in biological fluids and tissues such as whole blood, plasma urine, saliva, brain tissue, liver hair, muscle tissue. AAS can be used to in qualitative and quantitative analysis.

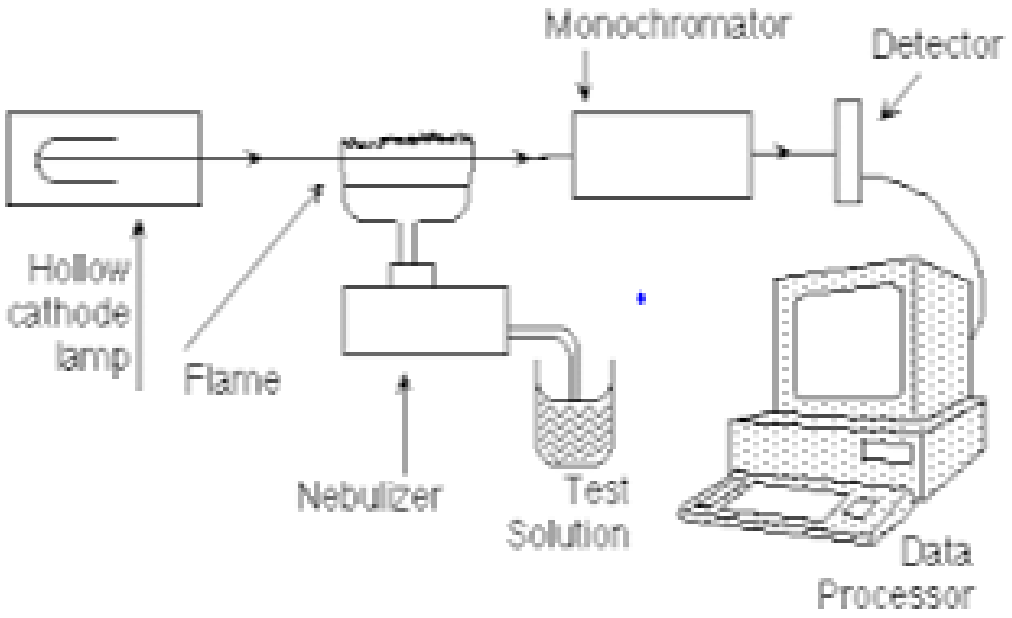

Figure 2: Schematic diagram of atomic absorption spectrometer

2.3.1 Basic Principles

The basic principle of both FAAS and ETAAS is that sample is introduced into the atomizer, where it is desolated and then atomized. The analyte atoms so formed then quantitatively absorb light in a way that is proportional to the concentration of the atoms of the analyte in the cell. The light, which is at a specific wavelength, is then isolated from other wavelengths that may be emitted by the atom cell and then detected. Thus, much of the instrumentation used for electro thermal and flame atomic absorption spectroscopy is identical. Both techniques require a similar source, background correction system, line isolation device (monochromatic or polychromatic), detector and readout system. In AAS, a solution containing the analyte is introduced into a flame. The flame converts samples into free ground state atoms that can be excited. A lamp emitting light at a wavelength specific to the atoms is passed through the flame, and as the light

energy is absorbed, the electrons in the atoms are goes to an excited state. The technique makes use of the atomic absorption spectrum of a sample in order to assess the concentration of specific analytes within it. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert law.

A=a*l*c Where A is absorbance l is path length of light through the sample cell and a is the absorptivity constant and c is concentration of the sample.

The absorptivity is the proportionality constant in Beer's low which varies from one light absorbing species to another and has wave length dependence, which is also different from one light absorbing species to another thus the value of A can be changed by one of the following ways for any given light absorbing species these are change the wave length ,change the path length , or change the concentration .The path length is usually fixed at some convenient value through spectrophotometer and sample cell design. Also, the absorptivity is usually specified only at, which is the wavelength at which light is most strongly absorbed by the light absorbing species. It is worth noting that at for a species of a given concentration in a cell of given path length the variation of A per unit change in concentration is most dramatic because absorptivity is greatest at *lamda max*. Therefore, at *lamda max* the concentration of a light absorbing species can be determined by comparing light absorption of solution of unknown concentration to the absorption of samples of known concentration. This is done by making a graph of A versus concentration for the "standards" or sometimes referred to as a Beer's Law plot or a standard curve and then finding what concentration corresponds to the value of an observed for the unknown.

2.3.2. Instrumentation

In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used are flames and electro thermal graphite tube atomizers. The atoms should then be irradiated by optical radiation, and a continuum radiation source could be an element Specific line radiation source or a continuum radiation source. The radiation then passes through a monochromatic in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector. The atomizers

most commonly used are (spectroscopic) flames and electro thermal (graphite tube) atomizers. The oldest and most commonly used atomizers in AAS are flames, principally the air-acetylene flame with a temperature of about 2300 °C and the nitrous oxide system (N_2O)-acetylene flame with a temperature of about 2700 °c Liquid or dissolved samples are typically used with flame atomizers. The sample solution is aspirated by a pneumatic analytical nebulizer, transformed into an aerosol, which is introduced into a spray chamber, where it is mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (< 10 μm) enter the flame. This conditioning process reduces interference, but only about 5% of the aerosolized solution reaches the flame because of it.

The processes in a flame include the stages of drying in which the solvent is evaporated and the dry sample Nano-particles remain, vaporization in which the solid particles are converted into gaseous molecule, atomization in which the molecules are dissociated into free atoms, and ionization where (depending on the ionization potential of the analyte atoms and the energy available in a particular flame) atoms may be in part converted to gaseous ions. In flame AAS a steady-state signal is generated during the time period when the sample is aspirated. This technique is typically used for determinations in the mg L^{-1} range, and may be extended down to a few μg L^{-1} for some elements

Graphite furnace atomic absorption spectroscopy (GFAAS) (also known as Electro thermal Atomic Absorption Spectroscopy (ETAAS)) is a type of spectrometry that uses a graphite-coated furnace to vaporize the sample. Briefly, the technique is based on the fact that free atoms will absorb light at frequencies of wavelengths characteristic of the element of interest (hence the name atomic absorption spectrometry). Within certain limits, the amount of light absorbed can be linearly correlated to the concentration of analyte present. Free atoms of most elements can be produced from samples by the application of high temperatures.

In GFAAS, samples are deposited in small graphite or pyritic carbon coated graphite tube, which can then be heated to vaporize and atomize the analyte. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. Applying the Beer-Lambert law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms (in graphite furnace AA). Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration. The main

advantages of the graphite furnace comparing to aspiration atomic absorption are the following: The detection limits for the graphite furnace fall in the ppb range for most elements, interference problems are minimized with the development of improved instrumentation and the graphite furnace can determine most elements measurable by aspiration atomic absorption in a wide variety of matrices.

GFAA spectrometry instruments have the following basic features: 1) a source of light (lamp) that emits resonance line radiation; 2) an atomization chamber (graphite tube) in which the sample is vaporized; 3) a monochromator for selecting only one of the characteristic wavelengths (visible or ultraviolet) of the element of interest; 4) a detector, generally a photomultiplier tube (light detectors that are useful in low-intensity applications), that measures the amount of absorption; 5) a signal processor-computer system (strip chart recorder, digital display, meter, or printer) **[37].** This technique has the advantage that any kind of sample, solid, liquid or gaseous, can be analyzed directly. Its sensitivity is 2–3 orders of magnitude higher than that of flame AAS, so that determinations in the low μg L^{-1} range (for a typical sample volume of 20 μL) and ng g^{-1} range (for a typical sample mass of 1 mg) can be carried out. It shows a very high degree of freedom from interferences, so that ETAAS might be considered the most robust technique available nowadays for the determination of trace elements in complex matrices.

I. Radiation source

We have to distinguish between line source AAS (LSAAS) and continuum source AAS (CSAAS). In classical LSAAS, as it has been proposed by Alan Walsh, the high spectral resolution required for AAS measurements is provided by the radiation source itself that emits the spectrum of the analyte in the form of lines that are narrower than the absorption lines. Continuum sources, such as deuterium lamps, are only used for background correction purposes. The advantage of this technique is that only a medium-resolution monochromator is necessary for measuring AAS; however, it has the disadvantage that usually a separate lamp is required for each element that has to be determined. In CSAAS, in contrast, a single lamp, emitting a continuum spectrum over the entire spectral range of interest is used for all elements. A high-resolution monochromator is required for this technique**.**

II. Hollow cathode lamps

Hollow cathode lamp (HCL) is the most common radiation source in LSAAS.A hollow-cathode lamp (HCL) is type of cold cathode lamp used in physics and chemistry as a spectral line source for atomic absorption spectrometers and as a frequency tuner for light sources such as lasers. An HCL usually consists of a glass tube containing a cathode, an anode, and a buffer gas (usually a noble gas). A large voltage across the anode and cathode will cause the buffer gas to ionize, creating plasma. The buffer gas ions will then be accelerated into the cathode, sputtering off atoms from the cathode. Both the buffer gas and the sputtered cathode atoms will in turn be excited by collisions with other atoms/particles in the plasma. As these excited atoms decay to lower states, they will emit photons. These photons will then excite the atoms in the sample, which will release their own photons and be used to generate data **[38].**

III. Electrode-less discharge lamps

Electrode-less discharged lamps (EDL) contain a small quantity of the analyte as a metal or a salt in a quartz bulb together with an inert gas, typically argon gas, at low pressure. Electrode-less discharge lamps provide high intensity (10-100 times) and narrow emission lines which lead to higher signal -to -noise ratio over the lines obtained using hollow cathode lamps. Types of light sources in Atomic Absorption Spectroscopy covered the essential features of the two commonly used light sources hollow cathode lamps and electrode - less discharge lamps. The benefits of electrode- less discharge lamps are realized especially when analyzing volatile elements like As, Sb, Bi, Cd, Hg, Rb, Sn, Te, etc. Sputtering of such metal atoms and their adsorption on cathode lamp side walls and windows begins to affect the useful life of the lamps. On the other hand electrode-less discharge lamps because of the high emission intensities overcome the problem easily and provide lower detection limits. Electrode-less discharge lamp with all their benefits are not as popular as hollow cathode lamps and are used mainly for analysis of about 15 volatile elements. The reasons are mainly higher cost and difficulty in operation in comparison to hollow cathode lamps **[39].**

IV. Wavelength Selectors

Wavelength selectors limit the radiation absorbed by a sample to a certain wavelength or a narrow band of wavelengths. Sensitivity of an AAS is improved when the bandwidths are narrow and detectability is improved when transmission is high. There are several types of wavelength selectors. Some of these are filters, grating monochromators, and prism monochromators. Filters are wavelength selectors that allow narrow bandwidths of radiation to pass through. They can be divided into four main categories: absorption filters, cut-off filters, interference filters, and interference wedges.

Grating monochromators are located within compartments of some AAS instruments and are responsible for producing narrow bands of radiation. There are five components found in most grating monochromators: an entrance slit, a collimating lens or mirror, a reflection grating, a focusing element, and an exit slit **[40].**

V.Detectors

A detector can be a mechanical, chemical, or electrical device that measures the change of a variable in its environment. In Atomic Absorption Spectroscopy, the amount of radiation that passes through a sample is measured and quantitatively described by transmittance. As light passes through a sample, power is attenuated as it is absorbed by the analyte in the sample. Transmittance, T, is the ratio of the source radiation's power exiting the sample, (P), to the source radiation's power entering the sample, (P_0). $T = P/P_0$

Transmittance can also be described as a percent, %T, when T is multiplied by 100. A large percent transmittance (approaching 100%) is characterized as a low analyte absorbance, whereas, a low percent transmittance (approaching 0%) characterizes a high analyte absorbance.

$A=-\log T$

Absorbance, A, can also be used to describe the reduction of electromagnetic radiation as it passes through a sample. Absorbance is a more common unit of measurement for AAS because of its linearity to analyte concentration with respect to Beer's Law **[41].**

3. Experimental

3.1. Materials and Method

Vernonia amygdalina (bitter leaves) was collected from two different areas (sites) of Gurage zone Sodo woreda using plastic bags. The sample was labeled properly, washed thoroughly with water to remove soil and other dirt's. Knife was used for cutting of sample. Oven and crucible were used for drying the samples. The samples were ground by mortar and pestle, the sample was weighed using analytical balance and transferred into volumetric flasks, HNO_3 and $HClO_4$ for digestion of samples were measured using measuring cylinder and they were added into the round bottomed flask. Digestion apparatus and round bottomed flasks were used for digestion of sample and blank. Highly pure chemicals, and distilled water were used for preparing solutions for analysis.

The analysis of *vernonia* sample was based on standard methods proposed by Certified Reference Materials (Ni, Cd, Zn, Cr and Cu) from Europe accredited lab. The selected heavy metals (Ni, Pb, Cd, Cr and Cu) were analyzed using flame atomic absorption spectrometric technique.

3.2. Description of the Study area

The capital town of Sodo woreda is named Buee. It is located in Gurage zone of the central Ethiopia region about 103 km from Addis Ababa, 40 km from Butgera and 150 km from hosanna, according to the recent report from woreda finance and economic development office (SWFEDO), the total population is estimated to be 200000 of which 91465 are males and 108535 are females [59]. The total area of the woreda is 88553.3 hectares. Its altitude is between 1900m to 2000 m above sea level (SWFEDO, 2015). Sodo woreda is divided into 54 kebeles and there are 4 towns. 90.6% of the population is dependent on farming while 9.4% lives in town engaged in different jobs. The selection of these sites was based on the availability of the plant and its popularity in using as medicinal plant by the local people around. Thus having reason, I have selected the stated area for our study.

3.2.1. Experimental study area

The research was conducted at Addis Ababa, the capital city of Ethiopia and the experiments were carried out from January 2022 to March 2023 in analytical chemistry lab.Department Addis Ababa University College of natural and computational science, Ethiopia.

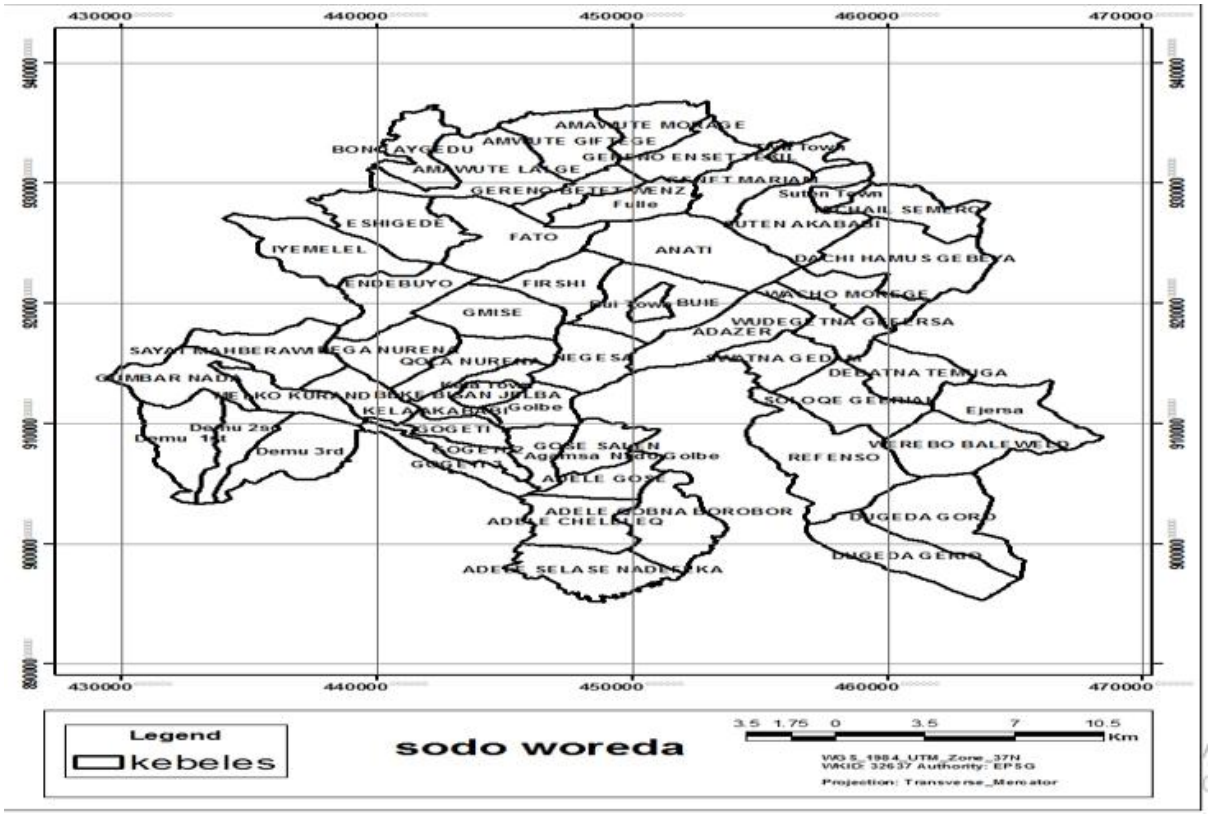

Figure 3: Map of sodo woreda.

3.3. Apparatus and Instruments

In this work different types of apparatus and instruments were used namely: oven (J. P.SELECTA, s. a), analytical balance (SCIENTECH, ZSA, 120), Whitman filter paper no. 42.digestion apparatus (Gollenhamp Kjeldahl apparatus), flame atomic absorption spectrometer (Analitikyjena, Model nov AA, 400p) and other common apparatus were used.

3.4. Chemicals and Reagents

The chemicals and reagents in the study were of high purity of grade Chemicals nitric acid, (69% HNO_3), and per chloric acid, (70% $HClO_4$) were used for digestion of vernonia amygdalina (bitter leaves) sample and blank. Standard solutions of the selected metals (Ni, Cr, Cu, cd and Pb) were prepared from 1000 mg/L stock solution of Certified Reference Materials from Europe accredited laboratory. Distilled and deionized water were used for rinsing and preparation of solution (samples).

3.5. Sample collection and Preparation

Vernonia amygdalina (bitter leaf) samples were taken from different sampling areas of buee town in plastic bags. The samples were transported to the laboratory for preparation. The sample was washed thoroughly using tap water, with detergents, and distilled water to remove soil and dirt from the outer surface of the samples. The samples were cut in to smaller pieces with a plastic knife; the samples were dried for one day an oven. The samples were ground using pestle and mortar, mixed together to get a representative sample and stored in plastic bags for digestion.

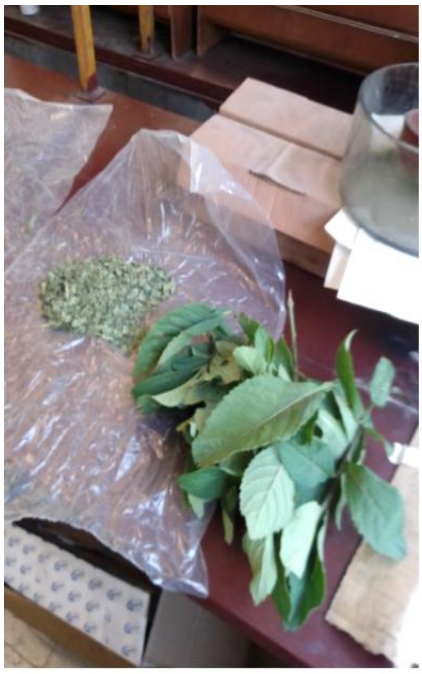

Figure 4: Leaves of V.amygdalina and sample

Figure 5. Powder of vernonia amygdalina leaves

3.6. Digestion of vernonia amygdalina (bitter leaves)

0.5gm triplicate samples were weighed using analytical balance (SCIENTEECH, ZSA, 120) and transferred to 250 mL round bottomed flask and 3 ml of 69% of analytical grade nitric acid and one mL of 70% per chloric acid were added. The mixture was heated at a temperature of 270℃ for 2:30hr on Gollenhamp Kjeldahl digestion apparatus. Using the same procedure this experiment was performed several times by varying the temperature at constant time, and varying the time at constant temperature. However, a temperature of 270℃ , 3:1 volume ratio of HNO_3 to $HClO_4$ acids respectively and a time of 2:30 hour was the optimized condition where clear colorless solutions were obtained. The solutions were cool twenty minute on the apparatus and five minute at room temperature. Finally, the mixture was diluted with 10 ml deionized water then filtered using what man filter paper no.42 in 50ml volumetric flask and diluted to the label mark with distilled water. The sample of *V.amygdalina* were digested in triplicate then labeled properly and kept for analysis. As the same procedure for digestion of samples three blanks were digested and each was analyzed for the required metals by FAAS.

3.7. Optimization of the working procedure

It is important to develop an optimum working procedure in order to get a reliable result from an analytical exeperment thus to preaper a clear colorless sample solution that is suitable for the analysis using AAS different working procedures for the digestion of Vernonia amygdalina leaves were assessed using the HNO_3 and $HClO_4$ acid mixtures by varing parameters such as volume of the acid mixture,digestion time and temperature. By examing the nature of the final digests obtained by varing the above parameteres, the optimized procedure was selected depending up on the clearness of the digestes ,less digestion time ,less reagent volume and simplicity for obtaining clear and colorless solutions of the resulting digests. The Optimization parameters for digestion procedure were 3:1volume ratio of acids at 270℃ and for 2:30 hours

Table 1: Digestion results for 0.5g leaf at different volume and constant temperature and time.

Volume ratio of acids (HNO_3 to $HClO_4$)	Temperature (^0C)	Time (hours)	Observation of experiment
3:1	270	3:00	Colorless solution
3:1	270	2:45	Colorless solution
3:1	**270**	**2:30**	**Clear colorless solution***
3:1	270	2:15	Colorless but not clear
3:1	270	2:00	yellowish color
3:1	270	1:30	Slightly yellow solution
3:1	270	1:00	Yellowish color

*Indicates optimum digestion time.

From the optimization results the digestion of the leaf has the ratio of Volume of HNO_3 to $HClO_4$ at 270℃ and 2:30 hrs indicated the clear colorless solution appeared at 2:30hr by varying time at lower time selected.

Table 2: Digestion results for 0.5g leaf at different temperature and constant volume and time.

Volume ratio of acids (HNO_3 to $HClO_4$)	Temperature (^0c)	Time (hours)	Observation of experiment
3:1	300	2:30	colorless clear solution
3:1	**270**	**2:30**	**colorless clear solution***
3:1	240	2:30	Yellowish color
3:1	210	2:30	Light brown.
3:1	180	2:30	Light yellow.
3:1	150	2:30	Colorless but not clear.
3:1	120	2:30	Yellow color

*Indicates optimum digestion temperature

From the optimization results of different temperature for digestion the leaf a temperature of 270℃ was selected since, at this temperature the procedure gave a clear colorless solution at lower temperature.

Table 3: digestion results for 0.5g leave different temperature and constant volume and time.

Volume ratio of acids (HNO_3 to $HClO_4$)	Temperature (^0c)	Time (hours)	Observation of experiment
4:1	270	2:30	Yellowish color solution
3:2	270	2:30	Colorless solution
3:1	**270**	**2:30**	**Clear colorless solution***
2:2	270	2:30	Pale yellow color solution
2:1	270	2:30	Yellowish color
2:3	270	2:30	Colorless but not clear
1:4	270	2:30	Slightly yellow color

From the optimization procedure 3:1, 270°c and 2:30hr were chosen for the digestion of clear and colorless and minimum volume.

*Indicates optimum volume ratio of reagents for digestion.

Figure 6: Digestion of V. amygdalina leaf photo taken during laboratory session

3.8. The operating condition of atomic absorption spectrometer

Intermediate standard solutions contain 10 mg/L were prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. these intermediate standards were diluted with distilled- deionized water to obtain four working standards for each metal of interest.in this study a total of 5 metals were analyzed using FAAS (Buck Scientific Puro-Graphic^{tm}) equipped with deuterium arc background corrector and air –acetylene flame system using external calibration curve after the parameters (burner signal intensity of the instrument. Three replicate determinations were carried out on each sample hallow cathode lamp for each metal operated at the manufacturer`s recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. All the five metals (Ni, Cd, Cu, Cr, and Pb) were analyzed by the absorption mode of the instrument.

The same analytical procedure was employed for the determination of element in 3 digested blank solutions. In this study sample digestion apparatus (Gollenhamp Kjeldahl) and Flame Atomic Absorption Spectrometry instruments were used. The digestion apparatus was optimized at a temperature of 270℃ for 2:30 hrs.

The operating condition for Flame Atomic Absorption Spectrometer is given in the Table 4.

Table 4: Instrumental conditions for metal analysis by FAAS

No	Element	Flame Type	Wavelength (nm)	Photo multiplier(nm)	Slit width (μm)
1	Pb	Acetylene gas	283	233	0.5
2	Ni	Acetylene gas	232	309	0.2
3	Cd	Acetylene gas	228	253	0.5
4	Cu	Acetylene gas	324	243	0.5
5	Cr	Acetylene gas	357	302	0.2

3.9. Stock Solution and Working Standards

For the metals analysis (Ni, Pb, Cu, Zn, and Cr), the standard containing stock solution (1000 mg/l) for each metal was obtained from Europe accredited laboratory. These stock solutions were used to prepare the intermediate standards (10 mg/l) and different concentrations of working standards. The intermediate standards and working standards are prepared from the stock solution by serial dilution with deionized water. After the working standards were prepared the instrument was calibrated to obtain good correlation between absorbance and concentration which is used to determine the unknown concentration of the sample.

3.10. Method Performance and Method Validation

A matrix spike is a type of quality-control sample used to evaluate the effects of sample matrices on the performance of an analytical method. Matrix spikes are used most often for quality control of organic-analyte samples because the analytical methods for organic-analyte samples involve extraction and analysis steps that can be affected by other chemicals in the sample (referred to as the sample matrix) **[42].**

Matrix Spiking is a technique that is used to evaluate the performance of an analytical procedure when testing a specific sample (matrix) type. In other words, a matrix spike test helps answer the question “Are we getting good (valid) results when we use this method to test this sample or

this type of sample?" A "good" matrix spike result increases our confidence in the accuracy and validity of the sample test results. Spike (MS) is generated A Matrix by adding a known amount (a spike) of analyte to a sample, testing the spiked sample, and determining if we have recovered the amount that we added. In practice, two portions of the sample are prepared for testing. In the "matrix spike" portion, we add a known amount of standard (to increase the concentration by a known amount). When we test the sample and then the matrix spike, the matrix spike result should be higher by that known amount added. If the analytical procedure is not working well for our sample, the matrix spike result will be higher or lower than we are expecting. A spiking solution is a standard that is chosen for preparing a matrix spike; the concentration of the analyte in the spiking solution is usually much higher than the concentration found in the unspiked sample **[43].**

3.11. Precision

Precision refers to how close two or more measurements are to each other, regardless of whether those measurements are accurate or not. It is possible for precision measurements to not be accurate. The precision of an analytical method is the degree of agreement among individual test results when the method is repeated to multiple samplings of a homogeneous sample **[44].** The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision is expressed as RSD of replicate results. The relative standard deviation of the samples is obtained as **[45]**: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous under the prescribed conditions.

$$\%\text{RSD} = \frac{\text{Standard Devation}}{\text{mean value}} \; x\ 100$$

3.12. Accuracy

Accuracy is defined as 'the degree to which the result of a measurement conforms to the correct value or a standard' and essentially refers to how close a measurement is to its agreed value. The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value **[45].** This is sometimes termed trueness. It is recommended that accuracy should be determined using a minimum of nine determinations over a minimum of the three concentration levels, covering the specified range (3 concentrations/3 replicates each of total

analytical procedures) **[46].** It is measured as the percent of analyte recovered by assay. The recovery can be determined by the equation **[47]:**

Accuracy (Recovery) = analytical result/true value x 100

3.13. Method Validation

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical application. The main objective of the validation is to demonstrate that the analytical method is suitable for its intended purpose, is accurate, and precise over the specified range that an analyte will be analyzed. Analytical Method Validation is to be performed for new analysis methods or for current methods when any changes are made to the procedure **[48].**

Spike-and-recovery and linearity-of-dilution experiments are important methods for validating and assessing the accuracy of analytical method for particular sample types. Spike-and-recovery is used to determine whether analyte detection is affected by a difference between the diluent used to prepare the standard solution and the sample matrix. Linearity of dilution refers to the predictability of spike or natural sample recovery for known dilution factors in the desired assay range. The two kinds of information are related, and experiments can be designed to test both spike recovery and delusional linearity simultaneously. In spike-and-recovery, a known amount of analyte is added (spiked) into the natural test sample matrix and its response is measured (recovered) in the assay by comparison to an identical spike in the standard diluent. The goal in assay development is to maximize signal-to-noise ratio while achieving identical responses for a given amount of analyte in standard diluent and sample matrix. The sample matrix may contain components that affect assay response to the analyte differently than the standard diluent. In spike-and-recovery experiment, a known amount of analyte is added to the sample matrix and standard diluent, and the two sets of responses are compared based on values calculated from a standard solution. If the recovery observed for the spike is identical to the recovery obtained for the analyte prepared in standard diluent, the sample matrix is considered valid for the assay procedure. If the recovery differs, then components in the sample matrix are causing the difference, and adjustments must be made to the method to minimize the discrepancy **[49].**

4. RESULTS AND DISCUSION

The results of the present study indicated that bitter leaves (Vernonia amygdalina) locally known as Grawa could be determined by using (FAAS), Flame Atomic Absorption Spectrometer ZEE nit 700P 150Z7P1025Tech: were used to determine the concentration of metals (Cd, Pb, Cr, Cu, and Ni) in vernonia sample. The qualities of the result obtained for analysis of heavy metals using FAAS are affected by calibration and standard solution preparation procedures. Calibration curves of the selected heavy metals were prepared to determine the concentration of metals in the sample solution. The instruments were calibrated using a series of working standards which were prepared from their intermediate solutions (10mg/l). The stock solution that is 1000mg/L of each metal was taken and 10mg/L was prepared as an intermediate for preparing different concentration working standards. The working standards were prepared according to the sensitivity of each lamp in the FAAS instrument. By using working standards, the instrument was calibrated with good correlation coefficient. After making sure the instrument was properly calibrated, the concentration of metals in each sample was measured and the calibration of each metal was drawn and presented in Figure 7 to 10.

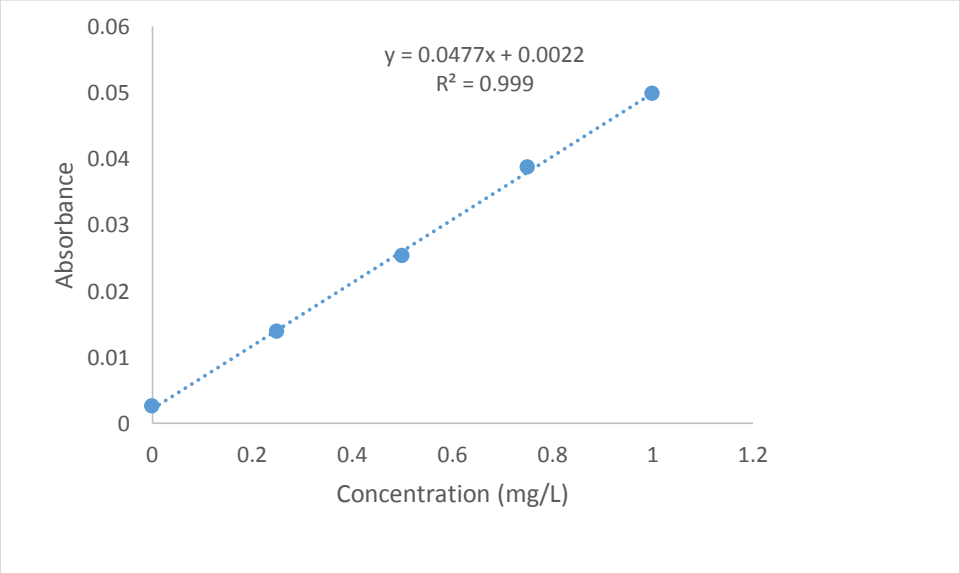

Figure 7 : Calibration curve of Nickel

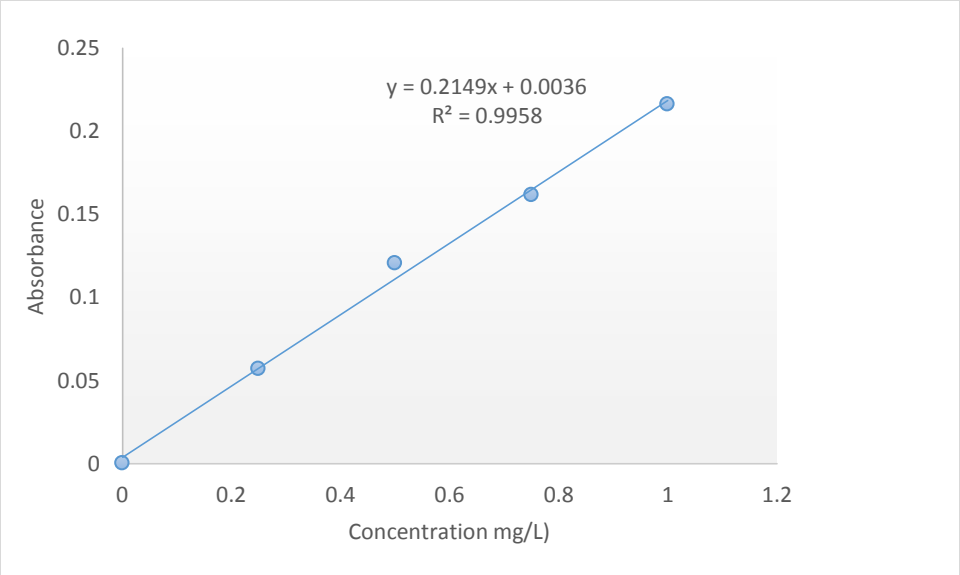

Figure 8 : calibration curve of Cadmium

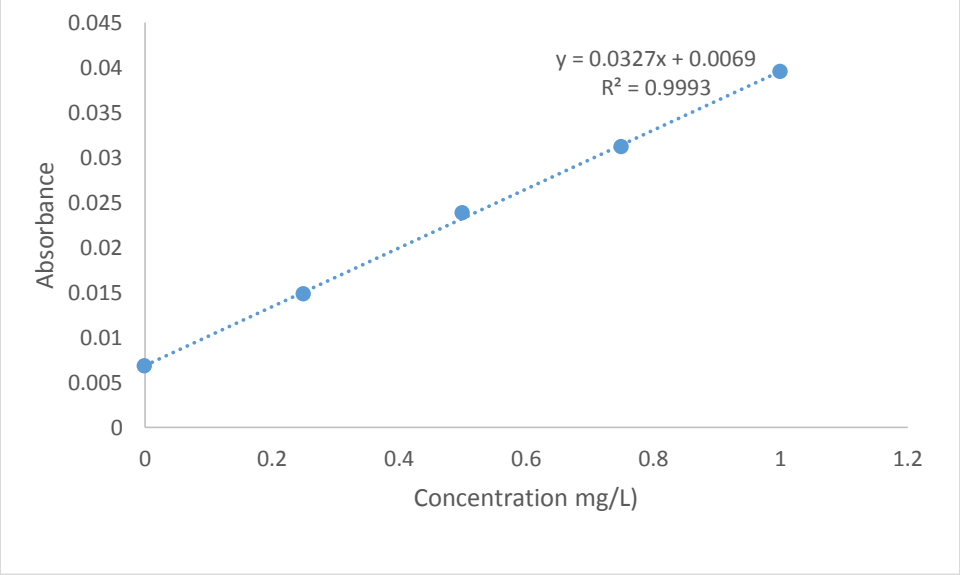

Figure 9: calibration curve of Lead

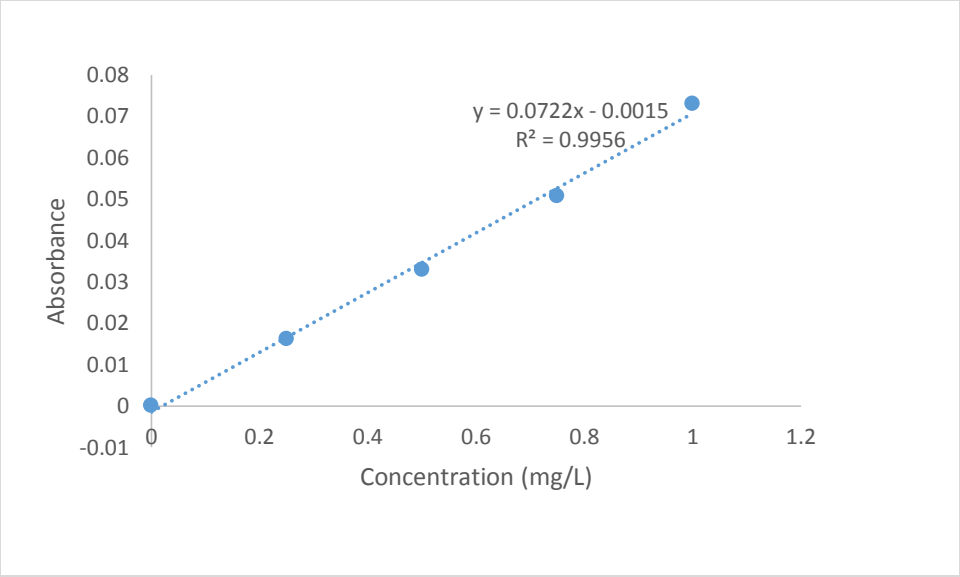

Figure 10: Calibration curve of Cupper

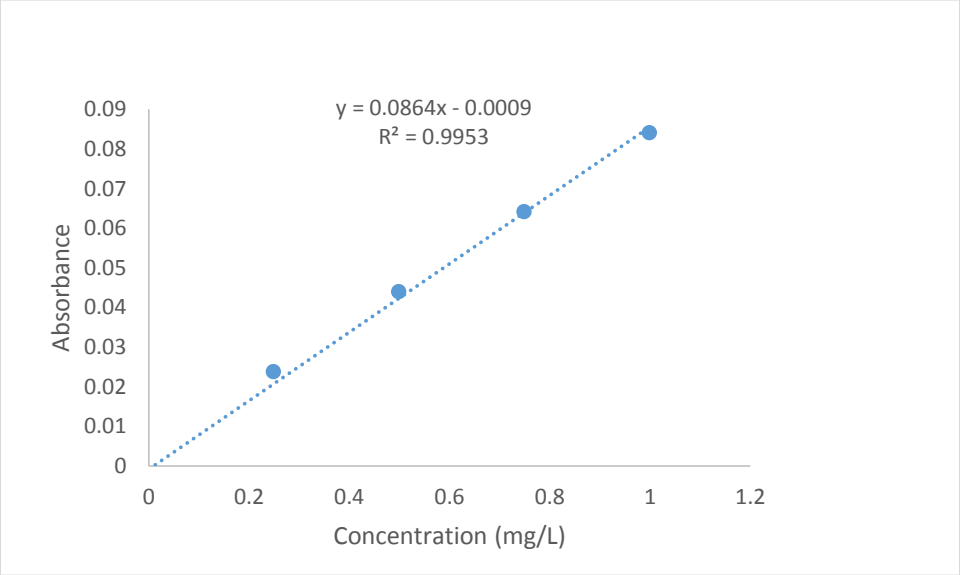

Figure 11: Calibration curve of Chromium

Table 5: Concentration of standard solutions used to calibrate the instrument and their corresponding correlation coefficients for the determination of metals

Metals	Conc. of stock solution (mg/L)	Conc. of Intermediate Solution (mg/L)	Conc. of standard series (mg/L)	Correlation coefficient
Ni	1000	10	0, 0.25, 0.5, 0.75,1	0.999
Cd	1000	10	0, 0.25, 0.5, 0.75,1	0.995
Cu	1000	10	0, 0.25, 0.5, 0.75,1	0.995
Cr	1000	10	0, 0.25, 0.5, 0.75,1	0.995
Pb	1000	10	0, 0.25, 0.5, 0.75,1	0.999

4.1. Method Detection Limit

Three blank samples were digested by following the same procedure as the samples and each of the samples were analyzed for metal concentration of Pb, Cd, Ni, Cu, and Cr by FAAS. The standard deviations for each element were calculated from the three blank measurements to determine method detection limit of the instrument.

The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence level that the measured concentration is distinguishable from method blank results." The method blank samples are used to calculate the MDL_b, which is a very similar calculation that also calculates the 99% confidence level that the result is derived from the sample rather from contamination/noise **[50]**.

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected. It can be determined visually, by signal to noise ratio, standard deviation of the response and the slope. Detection limit signal to noise approach can only be applied to analytical procedures which exhibit baseline noise. Comparing measured signals from samples with known concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. The method detection limit (MDL) may be expressed as: $DL=3\ \sigma/\ S$ where, σ is the standard deviation of the response, S is the slope of the calibration curve. The slope S may be estimated

from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, based on the standard deviation of the blank and the calibration curve. In this study method detection limit of each metal was established by digesting three analytical blanks for Vernonia. Each blank solution was also determined with FAAS as the same time and temperature as the Vernonia sample. The standard deviation of the blank was determined to calculate [51] .as can be seen from table 6, the method detection limit of each element is above the instrument detection limit.

Table 6: The mean value, standard deviation and MDL of the blank in Vernonia leaf samples.

Determined heavy metal	Blank concentration			Mean value	Standard deviation	MDL
	C_1	C_2	C_3			
Cd	0.0232	0.0062	0.0110	0.01346	0.007156	0.0216
Pb	0.03120	0.0252	0.0346	0.0336	0.00176	0.0528
Cu	0.054	-0.0061	0.038	0.029	0.025	0.076
Cr	0.0283	0.01469	0.015	0.0192	0.0064	0.223
Ni	-0.0195	-0.0237	-0.029	BDL	0.0037	0.236

Where C1, C2 and C3 are the concentration of the heavy metals in triplicate

Table 7: the mean value, standard deviation, and concentration and percent standard deviation of five selected heavy metals in the digested leaf sample

.

Determined heavy metal	Initial concentration			Mean value	Standard deviation (SD)	% SD
	C_1	C_2	C_3			
Cd	0.0221	0.01852	0.01661	0.019	0.003	14.6
Pb	0.3832	0.3991	0.4009	0.394	0.001	2.43
Cu	0.1914	0.1867	0.1879	0.189	0.003	1.25
Cr	0.0147	0.0144	0.0161	0.015	0.001	5.74
Ni	0.0671	0.0413	0.0205	0.043	0.024	54.8

Standard deviation (SD) is a measure of the spread or variability in the measured concentrations of a metal across multiple samples. A low SD indicates that the measurements are tightly clustered around the mean value, suggesting high precision in the FAAS measurements a high SD indicates greater variability in the measurements, which could be due to factors like sample preparation inconsistencies, flame stability or instrument sensitivity.

In the FAAS method, the observed SD %SD are crucial for assessing the quality of the measurements. Low %SD values, as seen in for Pb and Cu, suggest that the FAAS method was effective for these metals. However, higher % SD values, for Ni, indicate potential challenges in measurement precision, possibly due to low concentrations or other experimental factors.

The concentrations of Cd, Cu, Pb, Ni and Cr were measured in samples of *Veronia amygdalina* species growing in central Ethiopia, sodo buee. As heavy metal concentrations in plants depend mainly on their concentrations in the soil in which they grew or were cultivated. The results of the determinations of heavy metals in the analyzed plant samples are summarized in Table 2. Bearing in mind the diverse chemical properties of the various elements, each of them will be discussed in relation to its significance in terms of their essentiality and toxicity.

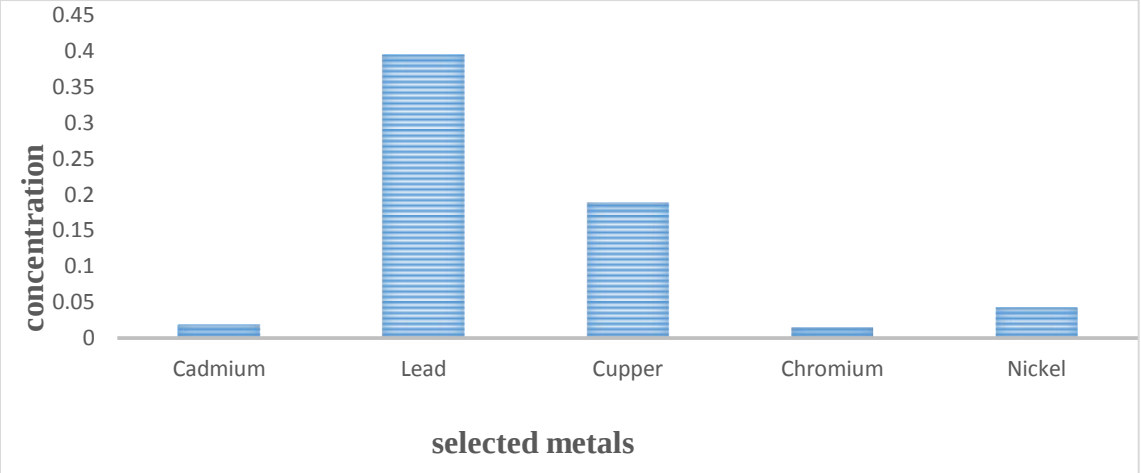

Figure 5: Concentration of the selected heavy metals (Cd, Pb, Cu, Cr and Ni) in vernonia leaves

4.2. Recovery test

Recovery studies involve the addition of a known of analyte to a sample and then determining what percent of the amount added is detected. The validity of the method was evaluated by spiking samples with standards of known concentrations and calculating percentage recoveries. Each level should be run in triplicate and the results averaged. The amount and percent of analyte recovered are calculated as follows:

Concentration recovered = test sample- baseline sample

% Recovery analytical result / True value x*100%* , or

$$\% R = \left(\frac{\text{spiked sample result} - \text{unspiked sample result}}{\text{known spike added concentration}}\right) x\ 100\%$$

The percentage recovery of metals is given below in Table 4. These values were within the acceptable range of 80-120% expected. So FAAS method was a good accuracy of analytical procedure.

Table 8: Recovery values of metals for the analyzed leaves sample.

Mean concentration (mg/L) ± SD				
Metals	Un-spiked Samples(mg/kg)	Spiked Amount(mg/kg)	Measured amount (mg/kg)	Percent recovery (%)
Cd	0.019	0.0380 ±0.003	0.0194	98
Pb	0.394	0.7902 ± 0.001	0.4301	92
Cu	0.189	0.3750 ±0.003	0.1980	94
Cr	0.015	0.0310 ±0.001	0.0156	96
Ni	0.043	0.2750 ± 0.024	0.2377	98

$$\% R = \left(\frac{\text{spiked sample result} - \text{unspiked sample result}}{\text{known spike added concentration}}\right) x\ 100\%$$

For example Cd = 0.038-0.019=0.019÷0.0194=0.979 x 100% =97.9%

The percentage recovery were found within the range 92-98% for the bitter leaves which is within the acceptable range for all analyzed heavy metal as shown in table 8. This indicated that the method is good accuracy.

Table 9: Permissible level of heavy metals by WHO in ppm

Cr	Pb	Cu	Cd	Ni
0.05	0.3	0.2	0.1 - 0.3	0.2

4.3. Heavy metals analysis in vernonia (bitter leaves) samples

Most of the heavy metal concentrations detected were lower than the acceptable levels established by the WHO and FAO.

Copper (Cu): There are several types of copper; the metallic form is less harmful, whereas the salt form is poisonous. High amounts of Cu can cause persistent anemia, vomiting, diarrhea, nausea, and abdominal pain

The mean concentration of copper (Cu) in this study was 0.1886 ± 0.00236 mg/kg, The Cu concentrations used in this study were below WHO standards.

Lead (Pb): Lead is the second most toxic heavy metal after arsenic in the top 20 list [53]. Lead in the environment is raised by several activities such as human and animal feces, phosphate fertilizer run-of, municipal effluent discharge, and mechanical workshops, especially lead storage batteries [54]. The mean concentration of lead (Pb) in this study was 0.3944 ± 0.0096 mg/kg, The Pb concentrations used in this study were comparable to WHO standards.

Chromium (Cr): Cr is an important micronutrient for proper plant and animal growth but, it is also considered a significant biological and polluting element. The mean concentration of Cr in this study was 0.0150 ± 0.00086 mg/kg the Cr concentration in this study was below WHO standard

Cadmium (Cd): Cd (II) does not have serious health issues for the communities. The major sources of Cd^{2+} include waste incinerators, agricultural soil run-off in the presence of phosphate

fertilizers, and Cd-based batteries. The solubility of suspended/bound Cd in water may increase as the acidity of the system increases [55]. Anthropogenic activities such as fossil fuel burning, mining, and metal production can increase the Cd concentrations in the environment [56]. The concentration of Cd in vernonia in this study was 0.019 ± 0.0028 mg/L. This value was less than the WHO allowable limit. This is in the line with the findings previous studies [62] of demonstrating that Cd concentration is not a serious problem in the vernonia sample under investigation. The permissible limit of cadmium in food and medicinal plants set by WHO, China and Thailand, was 0.3 mg/kg, [63]. The literatures suggested that it is safe for consumption if its level is less or equals to this permissible limit.

Ni is involved in fat metabolism and acid in fat deposition [57]. It also plays some role in body function including enzyme functions and occurs naturally more in plants than in animal flesh. It activates some enzymes systems in trace amount but its toxicity at higher levels is more prominent [58].

The mean concentration of Nickel (Ni) in this study was 0.0429 ± 0.023 mg/kg, The Ni concentrations used in this study were below WHO standards

Table 10: Concentration comparison of the analyzed heavy metals and the value of WHO Gide lines

Metals	Mean concentration of analyzed metals in this study in(mg/L)	Mean concentration of metals in WHO in (mg/L)	Reference
Cu	0.189	0.2	[52]
Cd	0.019	0.1-0.3	[63]
Ni	0.043	0.2	[52]
Cr	0.015	0.05	[63]
Pb	0.394	0.3	[63]

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The purpose of this study was the determination of some selected heavy metals (Cu, Cr, Cd, Pb, and Ni) in Vernonia amygdalina sample. The concentration of these heavy metals (Cu, Cr, Cd, Pb and Ni) were determined using FAAS. The result showed that the concentration of lead analyzed in vernonia amygdalina sample was relatively higher than the other analyzed heavy metals in the sample. And also vernonia amygdalina (bitter leaf) produced in sodo/buee were contains comparable level of lead with in the maximum allowable concentration of lead by WHO allowable limits however, the rest elements concentration was below the limit value, so this result may imply that consumers of this herbal medicinal plant may suggest that free from the harmful effects. The concentrations of these heavy metals analyzed in this bitter leaf taken from sodo/buee are safe for consumption as herbal medicine. Furthermore, and the efficiency of the digestion method was confirmed by percentage recoveries which were within accepted ranges, that is, eighty up to one hundred twenty.

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5.2. Recommendation

This study was performed in a very short period of time Due to this it was difficult to cover more sample site and collect larger sample size from all areas of sodo/buee and samples during all seasons to have more representative sample for the study. Therefore, it is recommended for other researchers to take more time and large number of sample to address of the limitation of this study. This work also did not study the heavy metal level of the soil, because vernonia *amygdalina* (bitter leaf) absorbs heavy metal ions and other minerals through their roots for their growth. Concentrations of heavy metals in the soil have its own effect on the concentration of metals in the leaf. So, it is recommended for other researchers to study concentration of heavy metals in the soil. Types of fertilizers, water for irrigation, manures, insecticides, herbicides, compost and weather condition of the growing area of vernonia amygdalina also have effects on the concentration of heavy metals in leaf. Therefore, it is recommended for other researchers to study the concentration of heavy metals in leaf in different weather condition of sodo/buee.

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