

ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF CHEMISTRY



Determination of Selected Heavy Metals in *Ocimum Sanctum* Sample Using
Flame Atomic Absorption Spectroscopy (FAAS)

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August 2016 / 2024
Addis Ababa, Ethiopia

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M.Sc. Thesis (Chem 692)

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A Thesis Submitted to the School of Graduate Program of Addis Ababa
University in Partial Fulfillment for the Degree of Master of Science in
Chemistry

August 2016 / 2024

Addis Ababa, Ethiopia

Declaration

I, the undersigned, declare that this research study is my original work under the supervision of my advisor in the college of Natural and Computational sciences, Department of Chemistry, Addis Ababa University and it has not been submitted in full or in part to in this or any other University. All sources of ideas and materials used for the project work are honestly acknowledged.

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This thesis has been submitted for examination with my approval as university advisor.

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Place and date of submission: School of Graduate Program , Addis Ababa University

August 2016/2024

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE PROGRAM

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Absorption Spectroscopy (FAAS)

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Acknowledgement

First and for most, I want to thank GOD for helping me all the difficult time of my life and giving me the strength to do my project.

I would like to express my deep and sincere gratitude to my advisor Dr. Nigussie Negash for his professional advice, encouragement and strong support. He supported this work from initial stage by read the first draft of the thesis and give me very valuable comments, correction and suggestion. I have a high respect and gratitude for his interest in this work.

Specially, I would like to express my deepest gratitude to Dr. Merid Tessema and Dr, W/Gebreal Yohannes for supporting and providing me chemicals and instrument for this work.

I would like to express my thanks to the Chemistry Department instructors who support me in any condition and my colleague, MSc study follow the programs.

I would also thanks to the Department of Chemistry, Addis Abeba University, for providing me this opportunity and giving me access to laboratory facilities for this work. I am also thankful to ministry of education giving me full scholarship for my M.Sc. study

Finally, I would like to thanks my family for their understanding, tolerance and exciting, support. And express my thanks to my little son Kirubel Mekidm for helping me by doing the housework throughout this project work

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Abbreviation

A	Absorbance
AAS	Atomic Absorption spectroscopy
CSA	Climate Smart Agriculture
DL	Detection Limit
EPA	Environmental Protection Agency
FAO	Food Association Organization
FAAS	Flame Atomic Absorption Spectroscopy
GFAA	Graphite Furnace Atomic Absorption Spectroscopy
HDL	High Density Lipoprotein
ICP – OES	Inductively Coupled Plasma – Optical Emission Spectroscopy
IARC	International Agency for Research Cancer
IQ	Intelligence Quotient
JECFA	Joint Expert Committee on Food Additives
MDL	Method detection Limit
PMT	Photo Multiplier Tube
PTWI	Provisional Tolerable Weekly Intake
Ppm	Part Per Million
ODS	Office of Dietary Supplement
OSHA	Occupational Safety and Health Administration

RDA	Recommended Daily Allowable
RDS	Relative Standard Deviation
SD	Standard Deviation
T	Transmittant
WHO	World Health Organization
XRF	X-ray Fluorescence

Abstract

Food additive plant have worldwide applications for its acceptability, aroma, color and sweetness of the food. Among the food additive plants of Ethiopia, *Ocimum sanctum* (in Amharic *Besobela*) is one of the well celebrated and most widely used home food additive mostly used during the preparation of pepper (berbere in Amharic) to improve the aroma and acceptability of pepper (berbere). The sampling technique used to carry out the analysis was purposive for the community in the selected area because the plant was widely used as food additive, the sample were selected from Bishoftu district in Gudino kebele. The aim of this study is to determine the concentration of selected heavy metal in holy basil (Besobela)sample. For the study 0.5-gram powdered sample that taken from Bishoftu district was digested by mixing 3ml of 69% HNO_3 and 1 ml of 70% perchloric acid, HClO_4 at the temperature of 270°C for 2:40'. The contents of the selected heavy metals (Cr, Pb Cu, Cd, and Zn) in holy basil were determined using FAAS and the obtained concentrations of these metals were compared with limit values set by WHO. The mean content of the selected heavy metals was determined from triplicate samples of holy basil, the mean concentrations of heavy metals analyzed in holy basil samples obtained in mg/l were; Cu(0.373 ± 0.151), Zn(0.832 ± 0.045), Pb(0.415 ± 0.0027), Cd(0.0288 ± 0.008) and Cr(BDL).The mean concentration of heavy metals obtained in holy basil were in the order of Zn> Cu>Pb> Cd and Cr was BDL. The concentration of lead analyzed in holy basil sample exceeded the maximum allowable concentration given by WHO. But the concentration of other metals Zn, Cu, Cr, and Cd were below the guideline set by WHO. The level of analyzed metals in this study are both higher and lower than the concentration of metals reported in different literatures.

Key words: - Holy Basil, Heavy Metals, Maximum Predictable Concentration, and Toxic Heavy

Metal

1. Introduction

1.1. Background of the Study

Spices are dried parts of plants, which have been used as dietary components of food often to improve its color, aroma, palatability and acceptability of food. Most of these are fragrant, aromatic and pungent (Mubeen et al, 2009). Spices may have other uses such as medicinal, religious ceremony, cosmetics, or perfumes production. (Pickersgill et al (2012) They consist of barks, leaves, fruits, seeds, and other parts of the plant (Aberie et al 2021). Natural food spices such as the holy basil contain a significant quantity of trace metals, these trace metals in spices and medicinal plants play an important role in living cells. (Belay et al 2015). Knowing the composition of trace metals in foods is important because of their essential or toxic in human health (Massadeh et al 2018). Heavy metals that are non-essential for our body even at a low amount are dangerous because they tend to bioaccumulation. Bioaccumulation means an increase in the concentration of a chemical in a biological organ through time, compared to the chemical's concentration in the environment (Speisky et al 1994). (Aberie et al 2021) The accumulation of heavy metals can have middle-term and long-term health risks, and strict periodic surveillance of these contaminants is advisable (Saranraj, et al 2014). Micronutrients are important in daily diets, because of their essential nutritious value and possible harmful effects (Peraza et al 1998). Metals like iron (Fe), copper (Cu), zinc (Zn), cobalt (Co) and manganese (Mn) are essential metals since they play an important role in biological systems (Jomova et al 2022). Trace metals may enter the food chain from the soil through mineralization by crops or environmental contamination as in the application of agricultural (Gupta et al (2019)) inputs such as pesticides, fertilizers or use of the polluted river for irrigation to water crops (Tsade et al 2016). An estimate suggests that about 13000 plant species worldwide are known to have been used as food additive and drugs. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds and so on (Prabhu et al 2012) That is any part of the plant may contain active components. (Pan, Si-Yuan et al 2013) The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant (Saranraj et al 2016) The medicinal actions of plants are unique to plant species or groups and are consistent with this concept as the

combination of secondary products in a particular plant is taxonomically distinct (Briskin et.al 2000)

1.2. Statement of the problem

Several elements are important for a wide range of biological processes due to their conjugated with proteins to form metallo proteins which are an important component in the enzymatic systems (Fraga, et.al 2005). The last three decades have seen increasing the production and consumption of spices in the world which is about 3.5% per year. This attributed to the accumulation of toxic trace metal, the spices may be contaminated with trace and heavy metals, and this could lead to health problems. , (Rucker et .al 2010). However, spices are one of the most widely used groups of antibacterial, antioxidant and anti-diabetic agents. On the other hand, information about the usefulness of heavy metals and safety of spices are limited (Soylak, et .al 2004). WHO has determined the maximum level for each toxic element in foodstuffs (Maris.s et .al 2012). The level of a heavy and trace metals in herbal spice beyond the permissible limit is a matter of great concern to public safety all over the world (Seddigi.et .al 2016). The problem is more pronounced in the cause of Ethiopia because most herbal spice are used by the society without realizing the concentration of toxic heavy metals as well as the trace metals. But there is no more literature (study) on the advantage and disadvantage of food additive in Ethiopia specially the level of heavy and trace metal in spice such as holly basil (*Ocimum sanctum* L.), however, the analysis of heavy and trace metal in ocimum sanctum was determined in Koladiba, Takusa and Chuati, north Gonder zone (north Ethiopia) only by using FAAS. So, this is one of a research gap. Therefore, based on the above presented condition is interested to this study because no study has been done in the study area. Thus, the current study focuses on the determination of the levels of heavy and trace metals in the flower and seed of holly basil (*Ocimum basilicum* L.), that is grown in Oromia Region, Bishotu district at Kebele 01 in order to protect the individuals from their adverse effects when used beyond the permissible limits, by using flame atomic absorption spectroscopy technique.

In order to determine the concentration of the metal such as Zn, Cu, Cr, Pb and Cd the atomic absorption spectroscopy technique was used. There are different technique for quantitative determination of heavy metal by AAS, such as flame in flame atomic absorption spectroscopy (FAAS); graphite furnace atomic absorption spectroscopy (GFAA); inductively coupled plasma-

optical emission spectroscopy (ICP-OES); X-rays in X-ray fluorescence spectroscopy (XRF). From this technique the Flame Atomic Absorption Spectroscopy (FAAS) is suitable technique for determining metals as parts per million (ppm) concentration levels with good precision for many elements. (Helaluddin et.al 2016) FAAS offers air-acetylene and/or nitrous oxide flame atomizer. FAAS technique provides fast analysis of 10-15s per sample, with very good precision (repeatability), moderate interferences that can be easily corrected, and relatively low cost (EH Evans et.al 2005).

1.3. Objectives of the Study

1.3.1. General Objective

- ❖ To determine the concentration of selected heavy and trace metals such as lead, cadmium, chromium, copper, and zinc in *Ocimum sanctum* L. using FAAS technique

1.3.2. Specific Objective

- ❖ Compare the concentration of the mentioned metals present in the flower and seed of *Ocimum Sanctum*. L with the permissible limits of WHO standard
- ❖ Compare the concentration of heavy metal in this study with those reported in the literature

1.4. Significance of the Study

Society has increasing interest in the therapeutic benefits of food additive. However, there is a wide spread misconception that natural herbs or spicy plants are inherently safe. There is also insufficient information available on the safety of traditional food additives and their products. Therefore, this study provides important information on the levels of selected heavy and trace metals in holy basil grown in the Oromia regional state around Bishoftu town that the society could be free of the potential health risks caused from the too much uptake of the heavy and trace metals in the herbal food additive. 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 results of this study could be used as reference for policy maker of the country who want to conduct the similar studies on human health and for other purpose

1.5 Limitation of the study

This study was conducted in short period of time that means the study is conducted summer in service program, the time is not suitable to study more sample size and lack of modern instrumental apparatus. However, for future it needs deep investigation about soil examination, water for irrigation, manures or compost and weather condition of the growing area of holy basil (*ocimum sanctum L.*) ,and so on, also have effects on the concentration of heavy metals on the sTherefore, the above reasons are the limitations of the study.

2. Review Literature

2. 1. Spices in Ethiopia

In Ethiopia, there are between 6,000 and 7,000 higher plant species, and out of which about 500 (about 8%) are edible. Almost all the food and drinks taken by Ethiopians daily consists of one or more spices, herbs, etc. Ethiopia's spices have a sort of mysterious nature, as they are not commonly found around the globe. Ethiopia is now mainly cultivating spices on a very small scale compared to other agricultural products. The systems are all rain fed and cultivated from the wild, hence one could say that almost all Ethiopian spices are organic. Most Ethiopian spices are cultivated as home gardens and from this sweet basil (*Ocimum basilicum* L) is most commonly used as spices (Aberie, et.al 2021)

2.2. Holy Basil

Holy basil belongs to the Scientific classification of *ocimum sanctum* Kingdom: *Plantae* Order: *Lamiales*, Family: *Lamiaceae* Genus: *Ocimum* Species: *O. tenuiflorum* with Binomial name of *Ocimum sanctum* L. Traditionally, holy basil (Besobela in Amharic) has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions. (Riyazi et.al (2020)) According to Manzoor IqbalKhattak and Khattak (2011), the content of Zn, Fe, Cu, Pb and Cd in *Ocimum basilicum*L. are (in ppm) 27.6, 242, 6.72, 0.26 and 0.25, respectively (Aberie, et.al 2021). The *Ocimum sanctum* L. has also been suggested to possess antifertility, anticancer, anti-diabetic, antifungal, antimicrobial, hepatoprotective, cardio protective, antiemetic, antispasmodic, and, analgesic actions (Prakash.et .al 2005). Many of the physiological benefits of holy basil can be attributed to its ability to assist with the body's internal housekeeping and protection of the body from toxin-induced damage. These functions are often attributed to holy basil's high content of phenolic compounds and anti-oxidant properties, with black/purple variety having a higher phenolic content and anti-oxidant capacity than white holy basil (Cohen.et.al 2014). Laboratory studies have shown that holy basil protects against toxic chemical-induced injury by increasing the body's levels of anti-oxidant molecules such as glutathione and enhancing

the activity of anti-oxidant enzymes. superoxide dismutase and catalysts, which protect cellular organelles and membranes by mopping up damaging free radicals caused by lack of oxygen and other toxic agents. (Srivastava. et.al 2021).

2.2.1 Physical Description

The holy basil plant Fig 1, is a small annual or short-lived, up to 1 meter (3.3 feet) in height. The stems are hairy and bear simple teeth. The fragrant leaves are green or purple, depending on the variety. The small purple or white tubular flowers have green or purple sepals and are borne in terminal spikes. The flower are nettles and produce numerous seeds.



Figure 1: - Plant of holy basil

2.2.2 Health Benefits of Holy Basil in Our Daily Life

Due to the presence of compounds like camphene, eugenol, and cineole, holy basil cures viral, bacterial, and fungal infections of the respiratory system (Umbreen et.al 2023). It can cure various respiratory disorders like bronchitis & tuberculosis and is also very good herb to cure asthma and other diseases. (Adesina et.al 2017) Holy basil acts as a mild diuretic & detoxifying agent and helps in lowering the uric acid levels in the body. Acetic acid and certain components in holy basil essential oil facilitate dissolution of the stones on the kidney (Chaudhry et.al 2019). If patient has a renal stone, it is suggested to take a mixture of juice of the leaves along with honey, every day for approximately six months so that it will remove the stone through urinary tract (Kaur, et.al 2020). Basil has a beneficial effect in heart diseases. Eugenol also proves to be useful in reducing cholesterol levels in the blood. Sweet basil cures all types of heart diseases and the “weakness” of the heart. Common-Diseases of children like common cold, high body temperature, loose stools and vomiting can be cured with juice of leaf. If blisters of chicken pox late in their appearance, sweet basil leaves given with kesar(saffron) will rush them (Murugan, et.al, 2021). Holy basil helps to kill bacteria and infections. Ocimum Sanctum helps treat skin infections both internally and externally Application of basil juice can help to treat fungal infection and other types of skin disorders (Yadava et.al 2024). It is one of the prime ingredients in the formulation of various medicines & home remedies. The anti-inflammatory properties of basil help promote eye health by preventing viral, bacterial and fungal infections. It also soothing eye inflammation and reduces stress. leaves dried under the sunlight and powdered, can be used in cleaning of teeth cure the teeth disorders. (Obi, Robert et al 2019) This also treats pyorrhea and other teeth diseases. Sweet basil is a natural mouth freshener and an oral disinfectant. It can also cure mouth ulcers. Sweet basil destroys the bacteria that are responsible for dental cavities, plaque, tartar, and bad breath, while also protecting the teeth. (Ahujan, et.al 2021)

2.3 Chemical Composition of holy basil

Phyto is derived from the Greek word which means plants. Phytochemicals are the chemical compounds which are present naturally in the plant parts and are used to prevent or cure any disease. More than 5000 photochemical have been discovered and yet many are still to be discovered (Kumer.et.al 2023). The chemical composition of the plant is quite complex, and proportion of the active chemical compounds differs in the different species of the genus. Basil

essential oil has major concentrations of eugenol (more than 70%) which is responsible for the anticancer, antioxidant and antimicrobial properties (Raina et al. 2013). Various chemical constituents containing biologically active compounds like 70% eugenol (volatile oil), 20% eugenol, methyl ether, caryophyllene, oleanolic acid, 3% carvacrol, caffeic acid, ursolic acid (triterpenoid), apigenin, luteolin, rosmarinic acid (phenylpropanoid) and chlorophyll have been isolated from the leaves (Mahajan et al. 2013). The leaves are rich in phenolic, terpenoids, volatile oils and fatty acid derivatives. The seeds contain mucilage; polysaccharide and the seed oil has fatty acids like stearic acid, palmitic acid, oleic acid, linoleic acid where linoleic acid is present in the major amount (Tangpao et al. 2018). Trace minerals like Zinc, Iron, and Manganese are present. Vitamin A and Vitamin C are present along with macro minerals like sodium, Calcium, phosphorus etc. (Godswill et al. 2020). The plants also contain saponins, alkaloids, tannins, glycosides, and traces of citric acid, tartaric acid, and maleic acid. The disparity in the physicochemical may be due to geographical or edaphic factors (Singh et al. 2021).

2.4. The effect of heavy metal on health

Periodic table consists of heavy metals to a notable portion with high density and atomic weight. Among them, the majorities are found in the biosphere, such as in water, soils, and rocks, and are also released into the surroundings from anthropogenic resources, mostly commercial and industrial. (Khalef, et al. 2022) However, recent experimental investigations show that some, including nickel, copper, and zinc, are vital for humans and are widespread in nature (Sarma, et al. 2011). Earlier studies have suggested that some common aromatic and medicinal crops might be capable of accumulating heavy metals from contaminated soil, suggesting the possibility that such plants could be used in the phytoremediation of contaminated soils. Cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) are some of the most widespread heavy metal contaminants of agricultural soils. (Lydakakis-Simantiris et al. 2016)

Contamination of water and air by toxic metal is an environmental concern and hundreds of millions of people are being affected around the world. Food contamination with heavy metal is another concern for human animal health. Concentration of heavy metal in water resource, air and food is assessed in this regard (Sama, et al. 2016). Metal among the other environmental pollutant may also occur naturally and remain in the environment. Hence, human exposure to metal is inevitable, and some studies have reported gender difference in the toxicity of metal.

They may frequently react with biological systems by losing one or more electrons and forming metal cations which have affinity to the nucleophilic site of vital macromolecules. Several acute and chronic toxic effect of heavy metal affect body organs. Gastrointestinal and kidney dysfunction, nervous system disorder, skin lesions, vascular damage, immune system dysfunction. Birth defects, and cancer are example of the complication of heavy metals toxic effect. Simultaneous exposure to two or more metals may have cumulative effect. High –dose heavy metal exposure, particularly mercury and lead, may induce severe complication such as abdominal colic pin, bloody diarrhea and kidney fairer (Anyanwu, et .al 2018). On the other hand, low –dose exposure is a subtle and hidden threat, unless repeated regularly, which may then be diagnosed by its complication, e.g, neuropsychiatric disorders including fatigue, anxiety and detrimental impact on intelligence quotient (IQ) and intellectual function in children. The fact that several metals have emerged as human carcinogens is another important aspect of the chronic exposure; while the exact mechanism is unclear, aberrant change in genome and gene expression are suggested as an underlying process (Mahurpawer.et al 2015).

Therefore, Heavy metals are among the most serious environmental contaminants in mining districts, 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. In Ethiopia, holy basil is grown in a wide range throughout the year in different area. Heavy metals are transferred from the abiotic environment to living organisms, accumulate in biota at different trophic levels, and therefore contaminate the food chain with implications to the human health and wildlife (Ali, et.al 2019). Heavy metals that presence in holy basil such as Zn, Cu, Cd, Pb and Cr is one of the most harmful health problems for human being due to its non-biodegradable nature and toxicity. (Verma, et.al 2017) Therefore, contamination of the food chain is one of the important pathways for the entry of these toxic contaminants in the human body which can cause health risks.

2.4.1. Cadmium (Cd)

Humans are exposed to cadmium with the resource of the use of inhalation and ingestion despite the fact that the number one health impacts recorded withinside the literature are thru nutritional exposure (kidney and bone damage) and inhalation from smoking tobacco and occupational exposure (lung damage). Dietary intake accounts for 90% of all exposure in non-smokers. (Bernard et.al 2008) Cadmium in the environment is toxic to plants and animals and many micro-organisms. Wastewater is key source of environmental cadmium contamination and diffuse pollution occurs

through industrial Air emissions and tremendous use of fertilizers on agricultural soils. (Ameer et.al 2011). Organic cadmium compounds are very unstable. In contrast to lead and mercury ions, cadmium ions are readily absorbed by plants (Shaari et.al2022). They are equally distributed over the plant. Cadmium is taken up via the roots of plant life to safe to eat leaves, end

result and seeds. During the growth of grains such as wheat and rice, cadmium taken from the soil is concentrated in the core of the kernel. (Almerud, et .al 2021) Cadmium also accumulates in animal milk and fatty tissues. Therefore, human beings are uncovered to cadmium whilst eating plant- and animal-primarily based totally foods. Seafood, such as mollusc and crustaceans, can be also a source of cadmium. Cadmium accumulates in the human body negatively affecting several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system. (Sarлак et.al 2022) Other damages that can be observed include reproductive, and development toxicity, hepatic, hematological and immunological effects. The Joint FAO/World Health Organization Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) has recommended as 0.007 mg/kg and below for cadmium (JEFCA, 2004). The EPA maximum contaminant diploma for cadmium in ingesting water is 0.005 of mg/L whilst the WHO observed the provisional guiding precept of 0.003 mg/L (WHO,2004a). (Mahurpawer et.al 2015) (Morais et.al 2012).

2.4.3. Copper (Cu)

Copper is a naturally-occurring metallic element that in soil at an average concentration of about 50 parts per million (ppm). It is present in all animals and plants and is an important nutrient for humans and animals in small quantities. The major causes of environmental copper releases include the mining, producing and purifying of copper, industries making products from copper such as wire, pipes and sheet metal, and fossil fuel burning. The major source of copper in intake water results from the leaching of copper from pipes and bath fixtures due to acidic water. Extra releases of copper to the environment include farming use against plant diseases and treatments applied to water bodies to eliminate algae. (Moskalyk et.al2003). Advantageous effects of copper is a component of several enzymes necessary for normal metabolic functions in humans. (Uauy, et.al 1998) The Recommended Daily Allowance (RDA) of copper for adults is 0.9 milligrams (mg/day), diet ranges from 1 to 1.6 mg/day, highest level of intake for an extended period of time

(chronic exposure) is 10 mg/day. (Taylor et.al 2023). Health effects Absorption/Metabolism Studies investigation oral absorption of copper have found the percentage absorbed ranging from 24-60 percent. The amount of dermal absorption is also not known, but a few studies indicate that it is very low (Myint et.al 2018), diet ranges from 1 to 1.6 mg/day. The safe highest level of intake for an extended period of time (chronic exposure) is 10 mg/day. Effects of copper shortage can consist of anemia, low numbers of white blood cells, osteoporosis in infants and children, and defects in connective tissue leading to skeletal difficulties. Short-term, but, acute effect in intoxication from ingestion of extreme copper can cause temporary gastrointestinal distress with signs such as nausea, vomiting, and abdominal pain. Liver poisonousness was realized in doses high enough that resulted in death. High levels of exposure to copper can cause destruction of red blood cells, possibly resulting in anemia. Long -term effects in mammals have efficient mechanisms to normalize copper stores in the body such that they are mostly protected from excess dietary copper levels. However, at high enough levels, chronic overexposure to copper can damage the liver and kidneys. Wilson's disease is a hereditary disorder in which copper builds up in the liver. Symptoms of liver toxicity (usually do not appear until adolescence. Carcinogenicity although some studies of workers exposed to copper have shown increased cancer risks (Hegazy et.al 2010).

2.4.3. lead (Pb)

Lead is a naturally occurring element in the earth's crust which is widely distributed throughout the environment in soil, water, and air by anthropogenic activities, elevating the natural levels. The lead content in the uppermost layer of soil can be characterized by atmospheric deposition from anthropogenic sources (Pattee, et.al 2000) Lead enters into the body through pathways like inhalation of wind-blown dust, ingestion of Pb contaminated soils, oral intake of Pb contaminated water, and food grown in Pb-contaminated areas. Pb accumulation in farm animal's tissues may additionally pose a main chance to human fitness via farm animals meat consumption. Pb is mostly bound to hemoglobin rather than RBC membrane after entering the cell. Histopathological observations showed that Pb ions are transported to the liver, in which they are able to set off continual harm to the liver. Pb toxicity additionally will increase blood enzyme degrees and decreases protein synthesis. Pb imposes toxic effects on kidneys through structural damage and changes in the excretory function (Kumar A et.al 2020). The other organ and tissue systems

affected due to lead toxicity are the nervous, cardiovascular, and reproductive systems. Children are particularly sensitive to this metal because of their rapid growth rate and metabolism, with critical effects in the developing nervous system (Rice et.al 2020). The Joint FAO/World Health Organization Expert Committee on Food Additives (JECFA) mounted a provisional tolerable weekly intake (PTWI) for lead as 0.025 mg/kg. The WHO provisional tenet of 0.01 mg/L has been followed as the same old for consuming water (WHO, 2004)

2.4.4. Zinc (Zn)

Zinc makes up about 75 ppm (0.0075%) of the Earth's crust, making it the 24th most abundant element. Sphalerite, which is a form of zinc sulfides is the most heavily mined zinc-containing ore because its concentrate contains 60–62% zinc. (Silwamba, et.al 2021).The human body contains 2–3 g zinc, and nearly 90% is found in muscle and bone. Other organs containing estimable concentrations of zinc consist of prostate, liver, the gastrointestinal tract, kidney, skin, lung, brain, heart, and pancreas. (Paun, et.al 2012). Zinc is an essential nutritional nutrient that performs essential roles at some stage in the whole body. Taking in an excessive amount of zinc may be harmful, however, and it could motive a variety of symptoms, which include nausea, diarrhea, and headaches. According to the Office of Dietary Supplements Trusted Source (ODS), an excessive intake of zinc can cause zinc toxicity. This toxicity can purpose gastrointestinal soreness and, while chronic, may additionally disrupt the stability of different chemical substances withinside the body, together with copper and iron. (Schoofs et.al 2024). Many over-the-counter, nutrient vitamins supplements, and cold remedies contain zinc. Taking multiple dietary nutritional dietary supplements on the identical time can placed someone vulnerable to exceeding their recommended nutritional allowance (RDA) of zinc. People may also experience toxicity from zinc in the environment., Zinc toxicity can be either acute, leading to short term side effects, or chronic, resulting in long term issues. The symptoms of acute toxicity will appear soon after taking a high dose of zinc and can include nausea, vomiting, stomachache, diarrhea, and headaches. If a person takes high levels of zinc over a long period, he/she can experience chronic zinc toxicity, which may lead to the following low levels of high-density lipoprotein (HDL), or “good,” cholesterol, (Dicken, et.al 2022)

2.4.5. Chromium (Cr)

Chromium (Cr) is a silvery white transition metal with atomic number of 24, relative atomic mass of 52.996 g/mol. At low concentrations, Cr is involved in natural human lipid and protein metabolism, so that very small amounts are needed for normal human life functions. Much of the daily intake of chromium, typically about 100 µg, is from foods such as grains, fruits and vegetables, potatoes, seafood, mushrooms and egg yolk. (Bielicka A et.al 2016) Chromium also occurs at much higher concentrations in industrial processes that can release potential pollutants to air and drinking water. (Bielicka A et.al, 2016) In dentistry, a variety of chromium-containing alloys can be found in most prosthodontic devices, orthodontic appliances, restorative devices, and in alloys and coatings of standard tools used by dentists. Chromium ions in solution occur in two common forms, relatively safer Cr (III) and more toxic Cr (VI), and particularly the latter is not similarly biocompatible as solid chromium oxide in human contact (Zohdi H et.al 2012) Chromium ions originating from antidiabetic drugs can be reduced and oxidized with certain metabolic pathways. In particular, Cr (III) from these drugs can be oxidized in the gastrointestinal medium, blood plasma, and other extracellular fluids into protein complexes and then ionized with DNA resulting in DNA adducts. (Aitio A et.al, 1984) Therefore chromium-containing anti-diabetic drugs can potentially have genotoxic and carcinogenic effects. In general, human exposure to chromium is mainly through inhalation, ingestion or skin contact. (Granam N et .al, 1996). Chromium-related cancer often occurs in the respiratory system, mainly as lung, nasal and sinus cancers. (Granam N et .al, 1996) Reaction of Cr (VI) with ascorbate (reductant) and hydrogen peroxide (oxidizer) will result in the accumulation of hydroxyl radicals that cause DNA damage, mutations, and increasing cancer potential. (Zhang X. et.al ,2011) Other symptoms may be more severe if the dose exceeds a threshold (Graham N et.al 1996) Mild symptoms: dizziness, general weakness, eye irritation Severe symptoms: kidney, liver, gastrointestinal, cardiac, hematologic or reproduction disorders, growth problems, nasal perforation, corneal injury. Abnormalities of teeth as discoloration and erosion, accompanied by high Cr concentration in tongue papillae.

2.5. Atomic Absorption Process

As the total of atoms in the light path rises, the amount of light absorbed also increases. By measure the amount of light absorbed, a quantitative determination of analyte can be made. The

use of different light sources and careful selection of wavelengths allow the specific determination of definite elements. A calibration curve is constructed by running several samples of known element concentration under the same conditions as the unknown. This enables the calculation of the analyte's concentration in the unknown sample. The quantity of interest in atomic absorption measurements is the quantity of light at the resonant wavelength which is absorbed as the light passes through a cloud of atoms. As the number of atoms in the light path increases, the quantity of light absorbed increases in a predictable way. By measuring the amount of light absorbed, a measurable determination of the amount of analyte element present can be made. The use of special light sources and attentive selection of wavelength allow the specific quantitative determination of specific elements in the presence of others. The atom cloud required for atomic absorption measurements is formed by supplying enough heat energy to the sample to dissociate the chemical compounds into free atoms. Pronouncing a solution of the sample into a flame aligned in the light beam helps this purpose. Under the suitable flame conditions, most of the atoms will go on in the ground state form and are capable of absorbing light at the analytical wavelength from a source lamp. The ease and speed at which precise and precise determinations can be made with this technique have made atomic absorption one of the most popular methods for the determination of metals. (Önal, et.al 2009)

Advantage of AAS technique; - Quantification of about 80 elements, detection limits may reach parts per billion, minimum sample size at least in μL . And, the use and applications of the technique through analysis of: -

- Biological sample hair nail etc.
- environmental sample air, water, soil, plants, animals etc.
- Industrial samples: Metal industries, Foods, Pharmaceuticals. etc.

General limitations of the technique: No information on chemical form of metal, Time-consuming sample preparation. Destructive. No multi-element analysis per run. the main components of the AAS Source of radiation such as Hollow Cathode Lamp and Electrode less Discharge Lamp; Cell or burner: - Flame, Graphite Furnace (Electro thermal or Flameless), Hydride Vapor Generator, Cold Vapor, Monochromator, Detector (Photomultiplier Tube), Readout system (Computer and Printer)

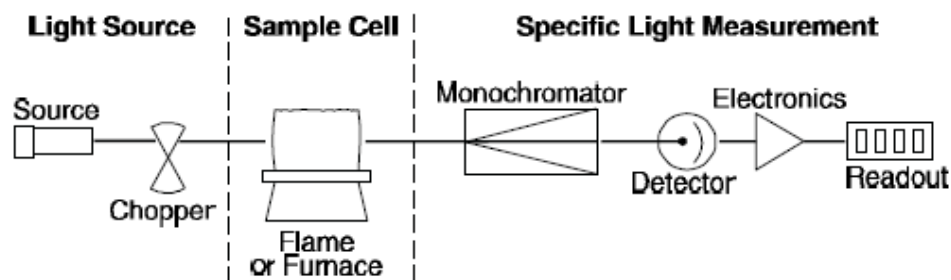


Figure-2.1. The main component of atomic absorption spectroscopy

a. Radiation source (Hollow cathode lamp)

This is the source of analytical light line for the element of interest and gives a constant and intense beam of that analytical line.

b. Atomizer (Flame): -The atomizer will destroy any analyte ions and break complexes to create atoms of the element of interest.

c. Wavelength selector (Monochromator)

A wavelength selector isolates analytical line photons passing through the flame and remove scattered light of the other wavelength from the flame. This only impinges a narrow line on the photomultiplier tube.

d. Detector (Photomultiplier tube (PMT))

It determines the intensity of the analytical line exiting the monochromator. The PMT is the most commonly used detector for AAS. (Butcher, et.al 2007)

2.6.1. Quantitative Analysis by Atomic Absorption

Flame atomic absorption spectrometer (FAAS) is a suitable technique for determining metals at parts per million (ppm) concentration levels with good precision for many elements. FAAS offers air-acetylene and/or nitrous oxide flame atomizer. FAAS system provides fast analysis of 10-15 s per sample, with identical or good accuracy (repeatability), moderate interferences that can be easily corrected, and relatively low cost. (Parsons, et.al 1997). The atomic absorption procedure is demonstrated in Figure 2.1. Light at the resonance wavelength of original intensity, I_0 , is focused on the flame cell containing ground state atoms. The original light intensity is reduced by an amount determined by the atom concentration in the flame cell. The light is then engaged onto the detector where the reduced intensity, I , is measured. The quantity of light absorbed is determined by comparing I to I_0 .

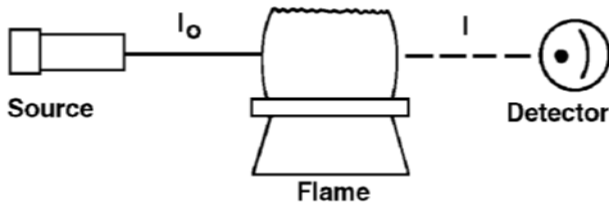


Figure 2.2 The atomic absorption process

Several associated terms are used to define the amount of light absorption which has taken place. The “transmittance” is definite as the proportion of the last intensity to the original intensity. $T = I/I_0$. Transmittance is an indication of the fraction of original light which passes through the flame cell to fall on the detector. The “percent transmission” is simply the transmittance expressed in percentage terms. $\%T = 100 \times I/I_0$. The “percent absorption” is the complement of out of a hundred transmission defining the percentage of the original light intensity which is absorbed in the flame. $\%A = 100 - \%T$. These terms are easy to visualize on a physical basis. The fourth term, “absorbance”, is morally a scientific quantity. $A = \log (I_0/I)$. Absorbance is the most suitable term for illustrating light absorption in absorption spectrophotometry, as this quantity follows a linear relationship with concentration. Beer’s Law defines this relationship as: $A = abc$; where “A” is the absorbance; “a” is the absorption coefficient, a constant which is characteristic of the absorbing species at a specific wavelength; “b” is the length of the light path intercepted by the absorption species in the absorption cell; and “c” is the concentration of the absorbing types. This calculation simply states that the absorbance is directly related to the concentration of the absorbing types for a certain set of instrumental conditions. (Jespersen, et.al 2006)

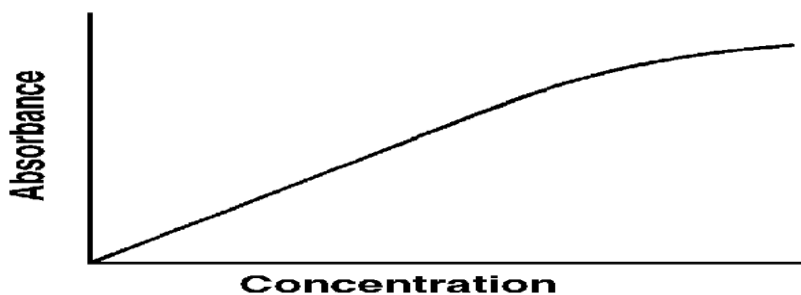


Figure-2. 3 Concentration versus absorbance

This direct related performance between absorbance and concentration is observed in atomic absorption. When the absorbance of standard solutions containing known concentrations of analyte are measured and the absorbance data are plotted against concentration, a calibration

relationship similar to that in Figure 2 is recognized. Over the section where the Beer's Law relationship is observed, the calibration produces a straight line. As the concentration and absorbance increase, non-ideal performance in the absorption procedure can cause a deviation from linearity, as shown in the figure. After such a calibration is recognized, the absorbance of solutions of unknown concentrations may be measured and the concentration determined from the calibration curve. In recent instrumentation, the calibration can be made within the instrument to provide a direct readout of unknown concentrations. Since the advent of microcomputers, precise calibration, even in the nonlinear region, is simple (Onal et.al (2009)

2.8. Accuracy

Accuracy is defined as 'the degree to which the result of a measurement obeys to the exact value or a standard' and principally mentions to how close a measurement is to its agreed value. In analytical chemistry, the term 'accuracy' is used in relation to a chemical measurement. The International Vocabulary of Basic and General Terms in Metrology (VIM) defines accuracy of measurement as "closeness of the agreement between the result of a measurement and a true value." The VIM reminds us that accuracy is a "qualitative concept" and that a true value is indeterminate by nature. In theory, a true value is that value that would be obtained by a perfect measurement. Since there is no perfect measurement in analytical chemistry, we can never know the true value. The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. It is recommended that accuracy would be determined using a minimum of nine determinations over a minimum of the three concentration levels, covering the definite range (3 concentrations/3 replicates each of total analytical procedures). It is measured as the percentage of analyte recovered by assay (Eisenhart 1969). The recovery can be determined by the equation.

2.6. Precision

The term precision is used to define the agreement of a set of results among themselves. Precision is usually stated in terms of the deviation of a set of results from the arithmetic mean of the set. There are systematic and random errors, the over-all error is the sum of the systematic error and random error. Since really random error is just as likely to be negative as positive, we can purpose that a measurement that has only random error is accurate to within the precision of

measurement and the more precise the measurement, this is the better idea of the true value, i.e., there is no bias in the data. In the case of random error only, good precision shows good accuracy. Several methodical errors can be repeated to a high degree of precision. Therefore, it follows that systematic errors prevent us from making the conclusion that good precision means good accuracy. When we go round the task of determining the accuracy of a technique, we are focusing upon the identification and elimination of systematic errors. Don't be misled by the statement that 'good precision is an indication of good accuracy.' Too many systematic errors can be repetitive to a high degree of precision for this statement to be true. Precision refers to how near or greater measurements are to every other, no matter whether or not the ones measurements are correct or not. It is probable for precision measurements to not be precise. The precision of an investigative method is the degree of agreement among specific test results when the technique is repeated to many samplings of a similar sample. The precision of an analytical process is generally expressed as the same old deviation or relative well-known deviation (coefficient of variation) of a sequence of measurements. Precision is articulated as RSD of replicate results. The relative standard deviations of the samples is obtained as (Shrivastava 2011)

$$\%RSD = \frac{\text{Standard Deviation}}{\text{mean value}} \times 100$$

2.7. Accuracy

Accuracy is defined as 'the degree to which the result of a measurement obeys to the exact value or a standard' and principally mentions to how close a measurement is to its agreed value. In analytical chemistry, the term 'accuracy' is used in relation to a chemical measurement. The International Vocabulary of Basic and General Terms in Metrology (VIM) defines accuracy of measurement as "closeness of the agreement between the result of a measurement and a true value." The VIM reminds us that accuracy is a "qualitative concept" and that a true value is indeterminate by nature. In theory, a true value is that value that would be obtained by a perfect measurement. Since there is no perfect measurement in analytical chemistry, we can never know the true value. The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. It is recommended that accuracy would be determined using a minimum of nine determinations over a minimum of the three concentration levels, covering the definite range (3 concentrations/3 replicates each of total analytical procedures).It is measured

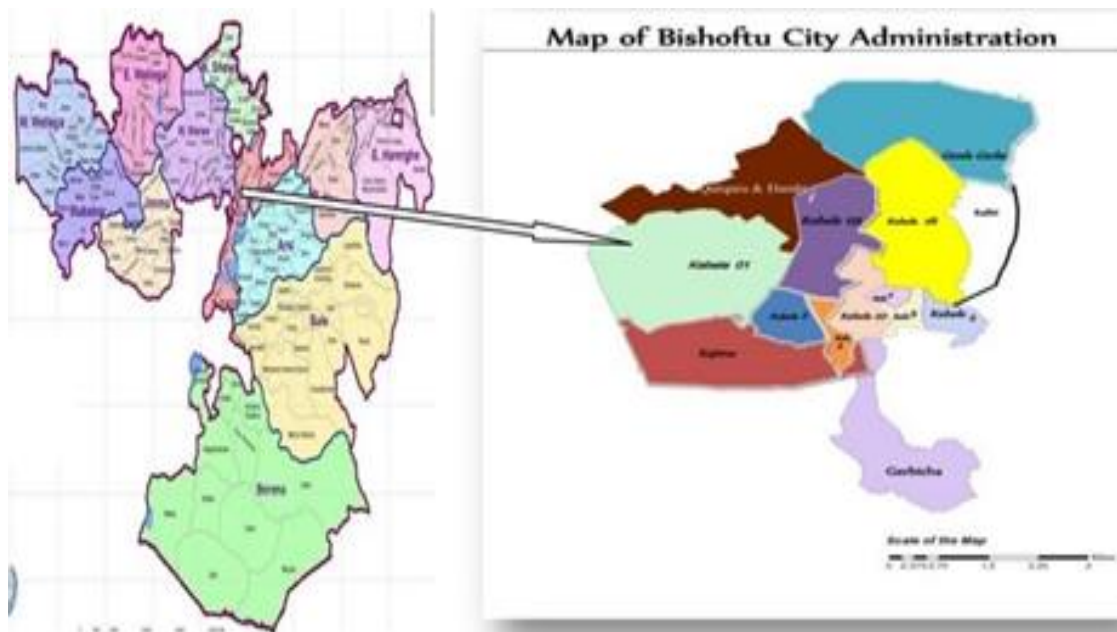
as the percentage of analyte recovered by assay (Eisenhart 1969). The recovery can be determined by the equation.

$$\text{Accuracy (Recovery)} = \frac{\text{Analytical Result}}{\text{True Value}} \times 100\%$$

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in kebeles 01 the smallest administrative unit in Ethiopia selected from Bishoftu district. Bishoftu is a town in central Ethiopia located in eastern Shewa zone which is found in Oromia Nations Regional state. It was also named as Debre Zeyte (mount of olives), however since known by the Oromo name Bishoftu, which was its name until 1995. The town serve as the primary airbus of the Ethiopia air force. The area is located 47.9Km. southeast of Addis Ababa between 8⁰45'N latitude and 8⁰75 E' longitude with geographical area of the city about 15,273 hectares (Brhanu et .al 2017). The Oreomia people are one of the indigenous people of Ethiopia who have their own culture, tradition, political legacy and kingdom. The study area lies at an altitudinal range between 1900 – 1995 above sea level and have agroecology of woynadega (mid altitude) and kola (low land) with a mean annual temperature of 23⁰C and annual rainfall of the district 968 mm according to the projected CSA final report of 2019.



Map of Oromia regional state

Figure -3.1: - sample area /study area of ocimum sanctum (holy basil)

3.2 Apparatus

The following apparatus were used in order to perform the project work. Different size beakers, measuring cylinders, micropipette, volumetric flasks, funnel, oven, filter papers, Stainless steel knife, Ceramic pestle and mortar were used for grinding and homogenizing the samples, Digital analytical balance was used for weight, round bottom flask (250mL) fitted with reflux condenser, microwave digester (Gallenhamp, England). volumetric flasks 50 ml. The selected heavy and trace metals was determined in the flower and seed of *Ocimum sanctum*. L under atomic spectroscopy as indicated in Figure 3.2, typical FAAS consists of radiation (energy) source, atomization compartment, monochromator, detector, and data readout system. (TT Dadebo et.al 2022)

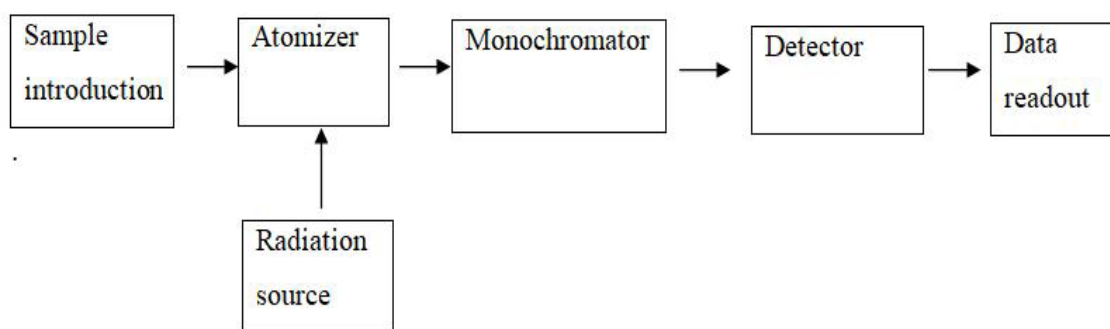


Figure 3.2: - Schematic diagram of FAAS equipment

3.3. Sample collection and preparation

The samples of fresh leaves of *ocimum sanctum* L were collected from local market, which are, in Bishoftu district from home garden fields, and collect the plant leaves in order to homogenize the sample. Samples were placed in plastic bags and labeled, and brought to the laboratory. Samples were first washed by tap water, then wash with distilled water, and first air-dried at room temperature and used oven dried at 50⁰C for 24hr for further drying and placed in dust free environment; then ground in to fine powder manually using a ceramic mortar and pestle and allowed to pass through a sieve. The powdered samples were placed in sample containers and stored in a cool dry storeroom preceding analysis. (Aberie et.al 2021)



a



b

Figure 3.3: Sample of holy basil, a) sample of holy basil before removal of leaves, and
b) holy basil after removed of the leaves.

3.4. Chemical and Reagent

All chemicals of high purity analytical grade reagents were employed. All reagents and chemicals used in the study were analytical grades. HNO_3 (69%) HClO_4 (70%) both of analytical grade and standard stock solutions containing 1000 mg/L, of each metal such as zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr) and copper (Cu) (buck scientific graphic) were used. HNO_3 were used for both extraction and acid digestion procedures. Stock standard solution with a concentration of 1000 mg/L was used to prepare intermediate or working standard solutions of 10 mg/L for the calibration standards of each metal ion salt. Deionized water was used throughout the study. (TT Dadebo et.al 2022)

3.5. Sample digestion

One of the basic requirements for sample preparation for analysis is to get the optimum condition for digestion. The optimum condition is the one which leads to minimum reagent volume consumption, short digestion time, minimum residue (clear solution), minimum or reduced digestion temperature and ease of simplicity. Optimization of the digestion procedure involved some changes of parameters such as reagent volume, digestion temperature and digestion time. Thus, by observing the nature of the solution in different procedures, the solution with the optimum conditions as indicated in Table 1 was selected for suitable analysis using FAAS. 0.5 gram of each of the powdered samples were weighed on a digital analytical balance and transferred into a 250 ml beaker. An optimized amount of the mixture of 69% concentrated nitric

acid (HNO₃), and 70% per chloric acid (HClO₄) was added to each plant sample according to the procedure that was optimized. The digested solutions were allowed to cool for 30 minut. To cool the solutions, deionized water was added to dissolve the precipitate that may be formed on cooling and gently swirled. The resulting solutions were filtered into a 50 ml volumetric flask with a Whatman filter paper number 110mm to remove any suspended and turbid matter. Subsequent rinsing of the filtrate with deionized water was followed until the volume reached the mark. For each bulk sample, triplicate digestions were carried out. The digested and diluted sample solutions were kept in volumetric sample bottles until analyzed by FAAS. (TT Dadebo et.al 2022)



Figure 3.4: - Digestion of *Ocimum sanctum* samples

Table- 1: Optimized conditions of digestion procedure for the sample of the leaves of *ocimum sanctum*

No	Wet (g)	Volume of reagents (mL)			Max time in °C	Time	Result
		HNO ₃	HClO ₄	Total			
1	0.5	5	3	8	270	3hr	Yellowish
2	0.5	5	2	7	270	3hr	Bright yellow
3	0.5	4	2	6	270	3hr	Light yellow
4	0.5	3	1	4	270	2:40'	Colorless and clear *
5	0.5	3	1	4	300	3:00	Colorless and clear
6	0.5	3	1	4	300	2:40'	colorless but turbid
7	0.5	3	1	4	240	3:00	Clear but cloudy
8	0.5	3	1	4	270	2:00	Clear but yellowish

As indicated in Table 1 eight optimization procedures were used to get the optimum digestion conditions. The procedures used in steps one through three were not chosen as optimum conditions for they used maximum reagent volumes and the highest digestion temperature as well as long time even if some of the procedures gave clear and colorless solutions. In steps five it give clear and colorless solution but it take long time and high temperature .in step six up to up to eight, the volumes of reagents were relatively low, and also give them a colorless solution but they are not clear , and also they took place at relatively long times and high temperature. Therefore, procedure four was chosen as optimum condition for the digestion, because it took place at relatively minimum reagent volumes, low digestion time to give clear and colorless

3.6. FAAS Operating Condition

Before analysis the sample of ocimum sanctum the blank solutions were prepared for calibrating the instrument, it was prepared by digesting the mixture of reagents following the same digestion procedure as the samples such as 3 ml of HNO₃ and 1 ml of HClO₄. The digestion of the blank was optimized at a temperature of 270°C for 2:40 hour, and diluted to 50 ml with deionized water. The operating condition for Flame Atomic Absorption Spectrometer is given in the Table 2. Measurements were made using the hollow cathode lamps for Zn, Cr, Cu, Cd and Pb at the proper wavelength and the slit width was adjusted using air acetylene flame. Instrumental conditions of experimental setup are shown in Table 2 and the working standards for determination of metals in spices samples using flame atomic absorption spectrometer. In this study sample digestion apparatus (Gollenhamp Kjeldahl) and Flame Atomic Absorption Spectrometry instruments were used

Table 2. Instrumental conditions for metal analysis by FAAS

No	Element	Flame Type	Wavelength (nm)	Photo multiplier	Slit width (nm)
1	Zn	Acetylene gas	213	394	0.7
2	Cu	Acetylene gas	324.	204	0.7
3	Cr	Acetylene gas	357	302	0.7
4	Pb	Acetylene gas	283.	233	0.7
5	Cd	Acetylene gas	228.	253	0.7

3.7. Stock Solution and Working Standards

For the metals analysis (Cd, Cr, Cu, Zn, and Pb), 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 working standards were 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. (Aberie et.al 2019)

4. RESULTS AND DISCUSSION

4.1. Instrumental Calibration

Flame Atomic Absorption Spectrometer was used to determine the concentration of metals (Zn, Cu, Cr, Cd, and Pd) in holy basil. The qualities of the result obtained for analysis of heavy metals using 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 (10 mg/l). The stock solution, that is 1000 mg/l of each metal was taken and 10 mg/l was prepared as an intermediate for preparing different concentration working standards, which was prepared from stock standard solutions, by diluting with deionized water to obtain five working standards for each metal of interest as indicated. 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, in table 3. The table also showed that the correlation coefficients of calibration curves of all five metals were closer to one. Thus, these results confirmed that there are strong linear relationships between two variables, which are absorbance and the concentrations of working standard solutions. After making sure the instrument was properly calibrated, the concentration of each element in the sample is equal to the quotient of the intercept and the regression coefficient of the calibration curve. Then, each metal content was calculated, and the results were reported as mean standard deviation the concentration of metals in each sample was measured and the calibration of each metal was drawn and presented in Figure 6.

Table 3. 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
Cr	1000	10	0.25, 0.5, 0.75, 1,1.5	0.959
Cu	1000	10	0.25, 0.5, 0.75,1,1.25	0.995
Pb	1000	10	0.25, 0.5, 0.75,1, 1.25	0.999
Zn	1000	10	0.25, 0.5, 1, 1.2, 1.5	0.997
Cd	1000	10	0.125, 0.25, 0.5, 1, 1.25	0.995

calibration graph of the selected metal

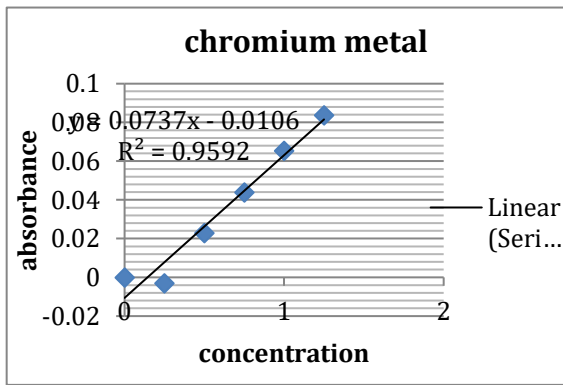


Fig 6a:- calibration graph of the chromium metal

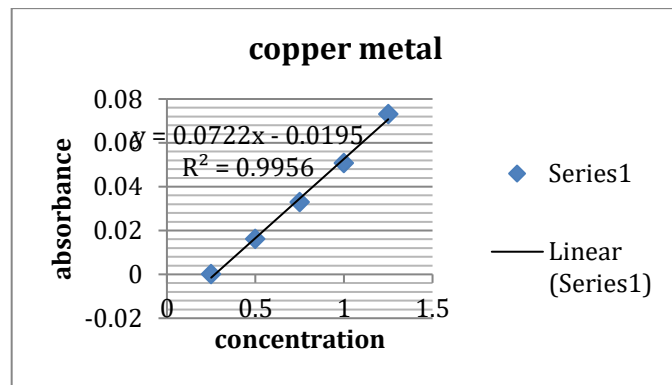


fig 6b:- calibration graph of the copper metal

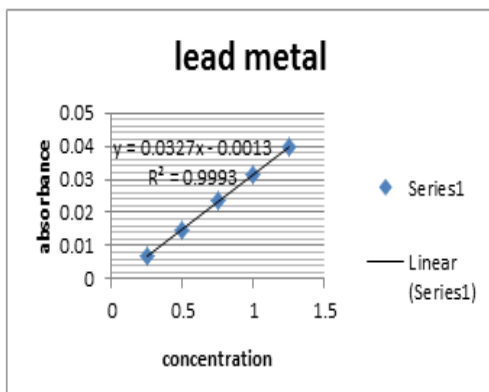


fig 6c:- calibration graph of the lead metal

Fig.6c calibration graph of lead metal

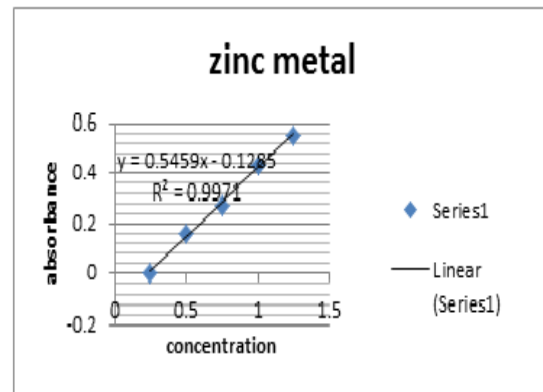


fig 6d:- calibration graph of the zinc metal

fig 6d. calibration graph of zinc metal

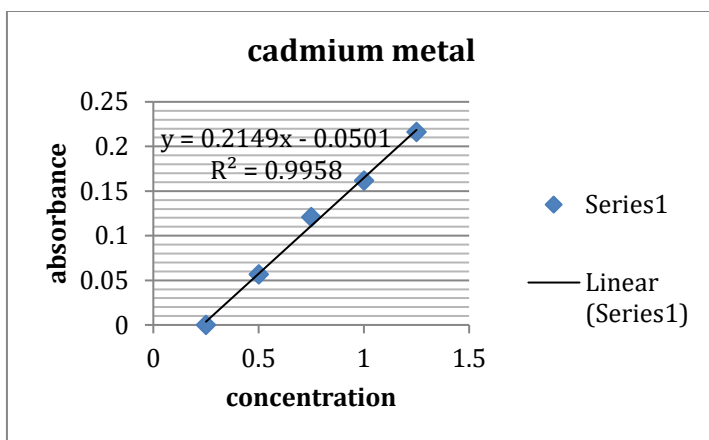


Figure 6e: - calibration graph of the cadmium metal

Figures 4.1. calibration graph of metals

4.2. Method detection limits (MDL)

The method detection limit (MDL) is the minimum concentration that can be detected by the analytical method with a given certainty. The limit of detection (LOD) is the lowest concentration level that can be detected statistically. In this study, the method detection limit of each metal was established by digesting three analytical blanks for *ocimum sanctum*. Each blank solution was also determined with FAAS at the same time and temperature as the holy basil sample. It can be calculated by multiplying the standard deviation of the reagent blank (Sample blank) by three ($MDL = 3 \times SD$ of sample blank).

Table 4. Method detection limit of metals

Metals	Zn	Cu	Cd	Cr	Pb
MDL(mg/l)	0.229	0.00696	0.0111	0.0381	0.0323

4.3. Determination of Heavy Metals in Holy Basil

The concentration of five selective heavy metals (Zn, Cu, Cr, Pb, and Cd) in the digested holy basil sample was analyzed by using FAAS. The mean \pm SD level of the analyzed metals obtained are given in Table 6.

Table 5. Mean concentration(mg/l) of heavy metals in Ocimum Sanctum sample

Metals	Zn	Cu	Cd	Cr	Pb
holy basil (mg/l) mean \pm SD	0.832 \pm 0.046	0.373 \pm 0.151	0.0288 \pm 0.008	BDL	0.415 \pm 0.003

Among the analyzed selected heavy metals given in Table 5 the level of chromium was below the method detection limit (MDL) in holy basil samples taken from Bishoftu, while the other selected heavy metals zinc, copper, cadmium, and lead were detected in holy basil sample.

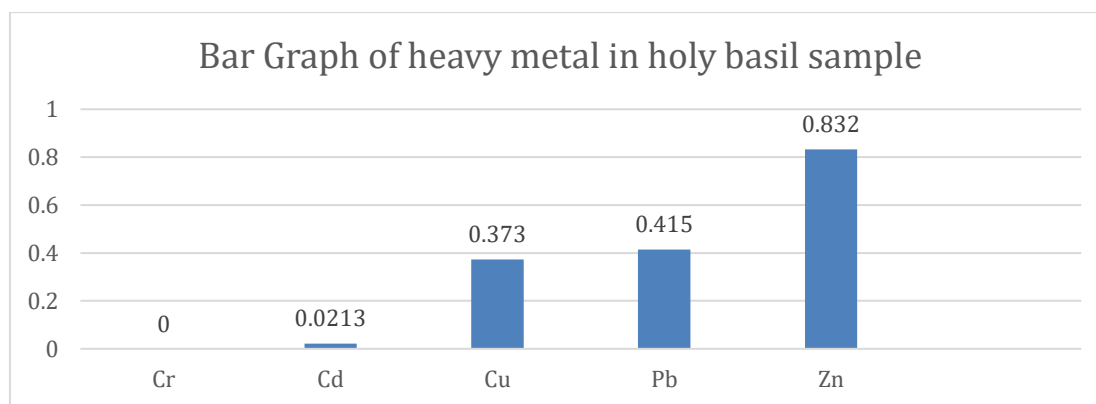


Figure 4.2. The concentration of the selected heavy metal in holy basil sample

4.4. Method validation

Method validation is the process of providing that analytical method is acceptable for its intended purpose. Due to the absence of certified reference material for leaves and seeds sample in the laboratory, the efficiency of the optimized procedure was checked by adding known concentration of each metal in 0.5g of Ocimum sanctum leaves' samples. The percentage of recovery is a crucial parameter for method validation. The spiked and non-spiked samples were digested and analyzed in similar condition. Then the percentage recovery of the analyst was calculated by:

$$\text{Percentage recovery} = \frac{C_{\text{minspicksample}} - C_{\text{minn0n-spicksamle}}}{\text{amountadded}} \times 100\%$$

Where, C_m = Concentration of metal of interest (Adapted from: IJRPC (2014), 4(1), 202-216)

4.5. 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:

$$\% \text{Recovery} = \left(\frac{\text{spiked sample result} - \text{un spiked sample result}}{\text{known spike added concentration}} \right) \times 100\%$$

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.

Table6. Recovery values of metals for the analyzed Ocmium Sanctum L. sample.

Mean concentration (mg/l) ± SD				
Metals	Un-spiked Samples(mg/l)	Spiked Amount(mg/l)	Measured amount (mg/l)	Percent recovery (%)
Zn	0.832± 0.046	0.332	1.15±0.022	95.78
Cu	0.373± 0.151	0.15	0.512±0.073	92.66
Pb	0.415± 0.003	0.166	0.567±0.066	91.56
Cd	0.0213± 0.008	0.00852	0.029±0.0021	90.37
Cr	BDL			

4.6. Comparison of Metals in Holy Basil with the Guideline set by WHO.

Heavy metal contamination of agricultural soils is a major environmental problem that can reduce both the productivity of plants and the safety of plant products as foods and feeds (PO Oladye, et.al 2022) Earlier studies have suggested that some common aromatic and medicinal crops might be capable of accumulating heavy metals from contaminated soil, suggesting the possibility that such plants could be used in the phytoremediation of contaminated soils. Cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) are some of the most widespread heavy metal contaminants of agricultural soils.

Metals obtained from the different sources are released in to the environment and tend to bio accumulate in plants, organisms and even biomagnified in the food chain where human beings are highly exposed. So, if holy basil is polluted with heavy metals, the metals will be transferred to animals and humans immediately and it affects human health if the concentration of heavy metals are above threshold limit.

Thus, the concentration of the selected heavy metals (Zn, Cu, Pb, Cd and Cr) obtained in this study were Zn (0.832 ± 0.046) mg/l, Cu (0.373 ± 0.151) mg/l, Cd (0.0288 ± 0.008) mg/l, Pb (0.415 ± 0.003) mg/l and Cr (BDL). The concentration of heavy metals in holy basil sample vary in the order of zinc > copper > lead > cadmium but the concentration of chromium is below the detection limit.

Cadmium (Cd)

Wastewater is key source of environmental cadmium contamination and diffuse pollution occurs through industrial air emissions and widespread use of fertilizers on agricultural soils. (Hayat et.al 2019)

Cadmium accumulates in the human body negatively affecting several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system. Other damages that can be observed include reproductive, and development toxicity, hepatic, hematological and immunological effects. (Ungureanu et.al 2022)

As indicated in Table 6, the concentration of cadmium was 0.0288mg/l. This result showed that there was no such much difference in other literatures showed that the concentration of cadmium

in other herbal medicines varied between 0.04 mg/L to 0.05 mg/L;(Dadebo, Eyob et.al 2022) for instance, in champion leaf, its concentration of cadmium was 0.0068 ppm (Baye et.al 2010). However, the concentration of cadmium in the study area was well below the permissible limit set by WHO and other organizations. The permissible limit of cadmium in food and medicinal plants set by WHO, China and Thailand, was 0.3 mg/l, (FAO/WHO, 2006). The literatures suggested that it is safe for consumption if its level is less or equals to this permissible limit.

Copper (Cu)

Beneficial effects of copper is a component of several enzymes necessary for normal metabolic functions in humans. (Uauyet.al 1998) The Recommended Daily Allowance (RDA) of copper for adults is 0.9 milligrams (mg/day), diet ranges from 1 to 1.6 mg/day. The safe highest level of intake for an extended period of time (chronic exposure) is 10 mg/day. (Taylor et.al 2023) As indicated in Table 5, the concentration of copper is 0.373mg/L. This result showed that there was no such much difference in other literatures showed that the concentration of copper in other herbal medicines varied between 0.19mg/L and 0.25mg/L;(Dadebo, et.al 2022). The regulatory limits of the WHO/FAO have not been established yet for copper in herbal medicines. However, China and Singapore in 2008 set the permissible limits of 20 mg/l and 150 mg/l, respectively. (Miraj et.al 2016) Thus, the concentration of copper in the studied herbal spice plant was well and below these limits.

Lead (Pb)

Lead is a toxic and non-essential metal having no nutritional value to living organisms. Lead enters into the body through pathways like inhalation of wind-blown dust, ingestion of Pb contaminated soils, oral intake of Pb contaminated water, and food grown in Pb-contaminated areas. Pb accumulation in cattle tissues may also stance a major risk to human health through cattle meat eating. After absorption, Pb is spread in the body through red blood cells (RBC). It was seen that in the work site, the level of Pb 0.415 mg /l (Table 5). From this, it could be observed that the level of Pb found in the study area was greater than the standard value given for Pb by WHO which is 0.3 mg/L in fruit and vegetable and 0.01mg/L in drinking water. (Dadebo, et.al 2022). This shows that high density found near the study area played a significant role in the level of Pb in the Bishoftu. From this it is possible to treat the soil or, and the irrigation

water that may has relatively high concentration of Pb, and its toxic effect on the human is very high, because Pb is very toxic heavy metal even at low concentration. Thus, the Bishoftu holy basil was not recommended for domestic use.

Zinc (Zn)

Zinc is an essential dietary nutrient that plays critical roles in the entire body. Taking in too much zinc can be toxic , however, and it may cause a range of indications, including nausea, diarrhea, and headaches. According to the Office of Dietary Supplements Trusted Source (ODS), an overdose intake of zinc can cause zinc toxicity. This toxicity can cause gastrointestinal discomfort and, when chronic, may also disrupt the balance of other chemicals in the body, including copper and iron (Lindsayet.al 2019).

Table 6 indicated that the concentration of zinc in the study area was found to be 0.872 mg/L. When compared to this concentration of zinc with other metals, (such as copper, lead and cadmium) the difference is great. But this result showed that there was no such much difference in other literatures showed that the concentration of zinc in other herbal medicines varied between 0.054 mg/L to 1.12 mg/L;(Dadebo et.al 2022). The permissible limit for zinc in food additive plants and herbal medicines set by WHO/FAO is 50 mg/l. Though there is little information about its toxicity, consumption of zinc beyond the permissible limit may result in toxic effect on the immune system (Waheed and Fatima, 2013). The concentration of zinc in the studied herbal spice plant was very much lower than permissible value set by WHO.

Table 7. Maximum allowable concentration of heavy metals by WHO.

Standard	Zn		Pb	Cr	Cd	reference
WHO (mg/l)	50m		0.3	0.05	0.1-0.3	Dadebo.et.al (2022)

The concentration of lead analyzed in the sample exceeded the allowable concentration by WHO. But the other metals' concentration was below the guide line given by WHO.

4.7. Comparison of Metals in Holy bail with Reported in the Literature

The concentration of the selected heavy metals Pb, Cd, Cu, Cr, and Zn) in holy basil has been reported in literatures, however with variations in effect of fertilizers, manures, water for irrigation used by farmers.

Table 8. Comparison of metal concentrations in mg/l with those reported in the literature

Country	Place	Zn	Cu	Pb	Cr	Cd	Methods	References
Algeria	Oran	0.38-2.5	0.6	0.26-0.52	-	0.13-0.16	AAS	(Zubairu.M.et.al 2020).
India	Raipur	18.7	18.7	7.5	10.7	-	ICP- AES	(Singhet.al 2010),
Iraq	Bagdad	0.842	0.402	0.127	0.06	0.015	AAS	(Jasim.et.al 2020)
Gahanna		1.02	0.11	-	-	0.08	AAS	Bempah.et.al 2012)
Ethiopia	Wolaita	1.21	0.2	0.1	0.1	0.04	FAAS	(Dadebo.et.al 2022)
Ethiopia	Oromia	0.837	0.373	0.415	-	0.0288	FAAS	This study

The mean concentration of zinc in the analyzed holy basil in this study was lower than those reported in Algeria (Zubairu. M et.al 2020). India (Singh et.al 2010), Ghana (Bempah et .al 2012) and Ethiopia (Dadebo et.al 2022) But the concentration of zinc almost is similar to the literature reported from Iraq (Jasim et al 2020).

The mean concentration of copper in the analyzed holy basil sample in this study was lower than the reported literature in Algeria (Zubairu. M et.al 2020). India (Singh et.al (2010)) and Iraq (Jasim et al 2020) But the level of copper is higher than those reported literature in Ghana (Bempah et .al 2012) and Ethiopia (Dadebo et.al 2022)

The mean concentration of lead in holy basil sample was lower than the reported literature in India (Singh et.al 2010). And the mean concentration of lead was higher than those reported in the literature in Algeria (Zubairu.et.al 2020), Iraq (Jasim et al 2020). and Ethiopia (Dadebo et.al 2022) but the mean concentration of lead was not analyzed in the literature of Ghana (Bempah et .al 2012)

The mean concentration of chromium in the literature in Algeria (Zubairu. et.al 2020), Ghana (Bempah et .al 2012) and Ethiopia (Dadebo et.al 2022) was detected. But the level of chromium

in India (Singh et.al 2010), Iraq (Jasim et al 2020) and in this study was not determine as shown in Table 8

The mean concentration of cadmium was analyzed in holy basil sample in this study was lower than those reported in the literature Algeria (Zubairu. et.al 2020)., Ghana (Bempah et .al 2012) and Ethiopia (Dadebo et.al 2022) and the mean concentration of cadmium was higher those reported in Iraq (Jasim et al 2020) But the concentration of cadmium was not analyzed those reported in the literature of India (Singh et.al 2010).

5. Conclusion and Recommendation

5.1. Conclusion.

The purpose of this study was the determination of some selected heavy and trace metals such as Zn, Cu, Pb, Cd, and Cr, in holy basil sample. The concentration of these heavy metals (Zn, Cu, Pb, Cd and Cr) was determined by using FAAS. In the present study, the analysis of heavy and trace metals in the selected spice plant was made by using FAAS by following the optimized digestion method. The digestion method was optimized by changing the parameters such as concentration, temperature and time until clear and colorless solution was obtained.

As indicated in Table 6, zinc had the highest concentration among the metals in the study and the metal with the next highest concentration was copper. The fact that zinc had the highest concentration among these metals was also true in other herbal medicinal plants based on different literatures (Baye et.al, 2010). But cadmium had the smallest concentration, which is 0.0288 mg/L. Among the analyzed selected heavy metals given in Table 6 the level of chromium was below the method detection limit (MDL) in holy basil samples taken from Bishoftu, while the other selected heavy metals are detected. The concentrations of all the selected metals in the herbal spice plant, *Ocimum sanctum*, sample were determined. The concentrations of Zn, Cu, Cd and Pb were 0.872, 0.373, 0.415 and 0.0288, (in mg/l) respectively, the concentration of zinc was the highest of all the metals. However, its concentration Zn, Cu, and Cd was not above internationally accepted permissible limits. But the concentration of lead in the study area is above the permissible limit in the spice plant under this study, the concentrations of some metals were below and the others were nearly equal to internationally accepted permissible limits but the concentration of lead was beyond the permissible limit so that this showed in the case of lead the plant was not safe for uses as food additives. Furthermore, the efficiency of the digestion method was confirmed by percentage recoveries which were within accepted ranges, that is, 80 – 120%.

5.2. Recommendation.

The schedule of the study was conducted is not suitable. Due to this it was difficult to cover more sample site from all areas of Bishoftu and to collect the 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 samples to address of the limitation of this study.

Holy basil was absorbing heavy metal ions and other minerals through their roots for their growth. Concentration of heavy metals in the soil have its own effect on the concentration of metals in the holy basil in the study area. This work also did not study the heavy metal level of the soil So, it is recommended for other researchers to study concentration of heavy metals in the soil.

water for irrigation, manures or compost and weather condition of the growing area of holy basil also have effects on the concentration of heavy metals on the sample. Therefore, it is recommended for researchers to investigate what kind of water source affect the concentration of heavy metals in holy basil, and to study the concentration of heavy metals in holy basil in different weather condition in Bishoftu district.

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