ADDISS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTEMENT OF CHEMISTRY

DETERMINATION OF SELECTED METALS IN TOSIGN LEAVES (*Thymus schemperi*) COLLECTED FROM BALE ZONE (OROMIA) BY FAAS

BY: BIZUAYEHU KEBEDE

Advisor: Dr MERID TESSEMA

ADDIS ABABA UNIVERSITY

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Determination of heavy metals in Tosign leaves (*Thymes schimperi*) collected from Bale zone (Oromia) by FAAS

MSc Thesis

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By Bizuayehu kebede

Adviser: Dr Merid Tessema

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Approval

As thesis advisor, I hereby certify that I have read this thesis prepared under my guidance and recommended that it can be accepted as fulfilling the thesis department.

	Signature	Date
Dr Merid Tessema (Advisor)	_____	_____

As members of the examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by **Bizuayehu Kebede Woldemariam** entitled: Determination of heavy metals in Tosign leaves(*Thymus schimperi*) collected from Bale zone (Oromia)" and recommended that it can be accepted as fulfilling the thesis requirement for the degree of Master of Science in Chemistry.

Approved by the Examining Board:

	Signature	Date
Dr Merid Tessema(Advisor)	_____	_____
Dr Solomon Mehretie (Examiner)	_____	_____
Prof. Chandravanshi (Examiner)	_____	_____

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Lists of Acronyms and Abbreviations

FAAS …… Flame Atomic Absorption Spectrometry

BDL …… Below Detection Limit

PMT…….. Photo MultiplierTube

SD ……. Standard Deviation

%RSD ……Percentage Relative Standard Deviation

WHO…… World Health Organization

FAO……Food and Agriculture Organization

MDL…… Method Detction Limit

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Abstract

The present study aimed at evaluating the elemental composition and daily dose standardization based on heavy metal presence in Tosign leaves (*Thyme schemperi*). Flame Atomic Absorption Spectroscopy were performed to analyze the presence of the heavy metals : copper, chromium, cadmium, lead and zinc. The mean contents of the selected heavy metals were determined from triplicate samples of thyme schemperi collected from Bale Goba In this study the concentration of heavy metals obtained inTosign leaves samples in mg/L were cadmium (0.018± 0.003), copper (0.188±0.002), lead (0.375± 0.009), zinc (0.853±0.051) and chromium (BDL). The concentration of Cd, Cu, Pb and Zn were below the maximum allowable concentration for medicinal plants given by WHO. Therefore, they were safe for health risk.

Key Words: thyme schemperi, metals, Flame absorption spectroscopy, Fenkel, Goba, Goro

CHAPTER ONE

1.Introduction

Ethiopia has a long-standing tradition of using plants as a primary source of medicine to treat a wide range of ailments. This practice is deeply rooted in the country's rich ethnic and cultural diversity, which is complemented by a unique array of flora and fauna. These natural resources have historically served as essential tools in addressing numerous health issues. Despite the advent of modern medicine, this indigenous health care system remains the primary source of care for an estimated 80% of the Ethiopian population. Remarkably, over 95% of traditional medicinal preparations in Ethiopia are derived from plants. The country boasts a diverse flora, with an estimated 6,500 to 7,000 species of higher plants, around 12% of which are endemic.

The Directorate of Traditional and Modern Medicine Research (TMMRD) within the Ethiopian Public Health Institute (EPHI) is actively engaged in operational and basic research focused on the quality, efficacy, and safety of traditional medicines. Additionally, the directorate investigates the factors contributing to drug resistance in addressing major health problems in the country.

Plants play a crucial role in the migration of chemical elements within natural ecosystems. Although plants exhibit biological selectivity in absorbing essential and toxic elements, allowing for some control over their chemical composition, various natural factors influence the element content in plants. These factors include soil type, climate, landscape, sunlight exposure, seasons, and human activities. Consequently, studying the elemental composition of the soil-plant system in conjunction with environmental conditions provides valuable insights [1, 3]. Variations in trace element concentrations can be used as indicators of specific plant growth conditions and the overall state of the environment [1, 4].

The determination of a wide range of elements in plants is essential for geoecological environmental monitoring, assessing the quality and safety of food and medicinal plants [5, 6]. and regulating the elemental content in the diets of humans, domestic animals, and poultry [7–9].

1.1 Description and distribution of *Thymus schimperi*

The Lamiaceae, also known as Labiatae[10] , is a large and diverse plant family, comprising approximately 236 genera and over 7,200 species[11]. This family is particularly significant in ethnomedicine due to its rich chemical composition [12], which includes compounds such as flavonoids, phenolic acids, terpenes, saponins, polyphenols, tannins, iridoids, and quinones[13]. Among the notable genera within the Lamiaceae family is Thymus, which is recognized for its numerous species and varieties [14].

In Ethiopia, the *Thymus* genus is represented by two indigenous species: *Thymus* serrulatus and Thymus schimperi [15]. Locally, these species are referred to as "Tosign" in Amharic and "Tesni" or "Thasne" in Tigrigna. These species are endemic to the Ethiopian highlands, thriving at elevations ranging from 2,200 to 4,000 meters above sea level [16]. *Thymus schimperi* is widely distributed across central, eastern, and northern Ethiopia, while *Thymus* serrulatus is predominantly found in the northern regions of the country. Specifically, *Thymus schimperi* is widespread in areas such as Bale, Shewa, Gonder, and Wollo, which are considered the primary growing regions in Ehiopia [17,34].

Globally, *Thymus* extracts have been traditionally used for various medicinal purposes. Orally, they are administered to treat dyspepsia and other gastrointestinal disturbances, bronchitis, pertussis, laryngitis, tonsillitis, and coughs associated with colds [18, 13]. Topically, thyme extracts are used to treat minor wounds, the common cold, disorders of the oral cavity, and as an antibacterial agent in oral hygiene [13]. These extracts have also been shown to possess antihelminthic [19], antibacterial, and fungicidal properties [20, 21, 22, 23].

In Ethiopia, the primary constituents *of Thymus schimperi* and *Thymus serrulatus* are thymol and carvacrol [24]. The pharmacological actions of thyme, particularly its medicinal benefits, are largely attributed to these phenolic compounds, especially thymol, which is a major component of their essential oils [16]. Besides their medicinal value, *Thymus* species in Ethiopia also have economic significance, serving as animal feed and bee forage [25].

1.2. Chemical composition of thymus schimperi

Medicinal plants are considered valuable resources for drug development due to their rich array of bioactive compounds. *Thymus schimperi* stands out as particularly rich in medicinally significant constituents, notably thymol and carvacrol. Research has shown that the essential oil extracted from T. schimperi grown in Ethiopia contains high concentrations of carvacrol (66.2%) and thymol (50%). These compounds are primarily responsible for the plant's potent antibacterial activity, effective against both gram-positive and gram-negative bacteria due to their impact on bacterial cell membranes [26, 27].

Many species within the *Thymus* genus yield commercially valuable *thyme* oil, which is renowned for its strong antimicrobial properties. The volatile oil extracted from thyme has been found to contain various active components, including p-cymene, γ-terpine, carvacrol, rosmarinic acid, eugenol, and thymol. This volatile oil exhibits a range of biological activities, including carminative, antiseptic, antimicrobial, and antifungal [28, 29] effects. Studies have suggested that the antimicrobial properties of *Thymus schimperi* may be attributed to the presence of compounds such as cardiac glycosides, anthraquinones, phenolic compounds, and steroids.

1.3. Ethno-medicinal and other uses of *Thyme schimperi*

The primary uses of thyme in culinary and food processing are defined by its components' aromatic and flavor-enhancing properties, as well as its antioxidant and antimicrobial activities. The thymol and carvacrol in thyme essence, alongside flavonoids and other polyphenols, contribute significantly to its antioxidant activity. Rosmarinic acid, hydroxycinnamic derivatives, and flavonoid compounds have shown important in vitro antioxidant activity, particularly in inhibiting iron-induced superoxide anion formation and lipid peroxidation in microsomal and mitochondrial systems. Additionally, thymol, a key component of thyme's essential oil, has demonstrated in vitro antioxidant activity by neutralizing the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical [30].

Thymus is an aromatic plant within the Lamiaceae family, widely used for medicinal purposes and as a spice across the globe. The non-medicinal uses of *thyme* are also noteworthy, especially in the food and aroma industries. *Thyme* is extensively utilized as a culinary ingredient and as a

food preservative, particularly due to its antioxidant effects. Both the ethanol extract and essential oil of thyme exhibit significant antifungal and antimicrobial activities, strongly inhibiting lipid peroxidation and effectively scavenging high-OH radicals [31]. The major phenolic components in thyme extracts, particularly thymol and carvacrol, exhibit higher antioxidant activity than the well-known antioxidants BHT (butylated hydroxytoluene) and α-tocopherol [26].

The antioxidative properties of thyme are significant in both medicinal and non-medicinal contexts. Numerous studies have shown that the essential oils and extracts of thyme possess strong antioxidative properties, including the ability to inhibit lipid peroxidation. Thymus schimperi, in particular, is rich in thymol and carvacrol, both of which are key constituents responsible for its antioxidant activity. Essential oil extracted from Thymus schimperi grown in Ethiopia has been found to be rich in carvacrol (66.2%) and thymol (50%), which are crucial for its antioxidative properties [32]. Antioxidants in food are capable of delaying, retarding, or preventing the development of rancidity and other forms of flavor deterioration due to oxidation. They extend the induction time, during which off-flavors develop, but adding antioxidants after this period tends to be ineffective in preventing rancidity.

Metal ions are the most significant pro-oxidants in foods, while antioxidants include compounds that act through radical scavenging, metal chelation, or other mechanisms [33]. Some plant extracts are recognized for their antimicrobial as well as antioxidant properties in food systems. Thyme is one of the most promising herbs for extracting natural antioxidants. Ethiopia has an abundant supply of wild thyme (*Thymus schimperi*), offering significant potential for the extraction of natural antioxidants and antimicrobials from this herb.

In Ethiopia, thyme leaves are widely used as a spice or additive to flavor a variety of food and beverage products. This extensive use underscores the importance of *Thymus schimperi* not only for its medicinal and economic value but also for its cultural significance in Ethiopian cuisine.

1.4. Economic values of *Thymus schimperi*

T. schimperi of food products and traditional medicines [34]. The fresh or dried leaves are used locally as condiments and tea [34], in the preparation of “berbere” (pepper powder) and “shirro” (bean/pea powder) [35], and for the preparation of Metata ayb (fermented cottage cheese) [36].

In traditional medicines, T. schimperi is used to treat different illnesses like gonorrhea, cough and liver disease, renal diseases, hypertension [34], stomach pain [37], kidney problems [38], and dermal fungi [39].

T. schimperi consists of approximately 1.0% to 2.5% of volatile oil [40]. Essential oils are volatile, natural, and complex chemicals with a strong odor that are synthesized as secondary metabolites by aromatic plants. Steam or hydroxo distillation is commonly used to obtain essential oils [41, 42]. There are about 3000 essential oils known today, with 300 of them being commercially relevant, particularly in the medicinal, agronomic, food, sanitary, cosmetic, and perfume industries [43].

The composition of the volatile oil varies depending on the chemo type of the plants [44].The principal components of T. schimperi essential oil are thymol and carvacrol (up to 64% of oil), along with linalool, p-cymol, cymene, thymine, a-pinene, Apigenin, luteolin, and 6-hydroxyluteolin glycosides, as well as di-, tri-, and tetra-methoxylated flavones have also been detected in T. schimperi leaves [40]. Although *T. schimperi* essential oil has antimicrobial activities [44], the leaves of T. schimperi are commonly used by locals as a food preservative, medicine for various ailments, and food flavoring and seasoning [45]. Pregnant mothers in Ethiopia are using *Thymus schimperi* by tea. Essential oil constitutes the plants is commonly used when fresh herbs are boiled in tea.

The results of the developmental toxicity test [46] show that administration of a high dose (260 mg/kg) of the essential oil of T. schimperi caused significant delays in fetal and embryonic development, a decrease in the number of implantation sites, and an increase in resorption number, suggesting its developmental toxicity. Similarly, higher doses of *T. schimperi* essential oil resulted in a significant reduction in maternal weight gain, placenta weight, and litter weight. Furthermore, at the middle (130 mg/kg) and high doses (260 mg/kg) of the essential oil extract, there was significant retardation in the development of the otic system, olfactory system, and branchial bars. Therefore, pregnant women should have a general awareness that risks could be associated with taking the essential oil during pregnancy, and consuming too much T. schimperi during pregnancy may be harmful [47].

1.5. Levels of trace metals in plants

The presence and concentrations of various elements in different plant depend on the composition of the soil, water and fertilizers used as well as permissibility, selectivity and absorbability of plants for the uptake of these elements. Hence, the observed variations in concentration of the elements are attributed to the nature of the plant as well as its surroundings [48].

The elements Fe^{+2}, K^{+}, Mg^{+2}, Na^{+}, Ca^{+2}, Co^{+3}, Mn^{+2}, Zn^{+2} and Cu^{+2} have been classified as essential elements, Ni^{+3}, Cr^{+3} are possibly essential while Cd^{+2}, Pb^{+4} and Li^{+1} are non-essential elements for the human body. Among the various elements detected in different medicinal plants used in the treatment of different diseases. It is interesting to note that some of the medicinal plants used by local physician and common people have high concentration in the range of ppm of Mn^{+2}, Fe^{+2}, Cu^{+2} , Zn^{+2} etc. The concentration of K^{+} and Ca^{+2} are in the percentage level. Zn^{+2} is important in wound healing and also functions as an antioxidant. Mn^{+2} are essential for normal functioning of central nervous system and are a good anti-oxidant.

Essential trace elements of the human body include zinc (Zn), copper (Cu), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), and molybdenum (Mo). Although these elements account for only 0.02% of the total body weight, they play significant roles, e.g., as active centers of enzymes or as trace bioactive substances. A major outcome of trace element deficiencies is reduced activity of the concerned enzymes. However, since each trace element is related to so many enzymes, deficiency of a single trace element is often not associated with any specific clinical manifestations, but rather manifests as a combination of various symptoms. Because of the presence of trace elements in very small amounts and the absence of specific clinical features associated with their deficiency, it is often difficult for clinicians to identify deficiencies of some particular trace elements. (Professor Emeritus, University of Tokyo).

1.6. Essential , non-essential metals and human health

Metals play important roles in a wide variety of biological processes of living systems. Homeostasis of metal ions, maintained through tightly regulated mechanisms of uptake, storage and secretion is therefore critical for life and is maintained within strict limits [49, 50]. Metal ion

transporters participate in maintaining the required levels of the various metal ions in the cellular compartments [51].

It is also known that several essential transitional metals, such as zinc, iron, copper, cobalt and manganese participate in the control of various metabolic and signaling pathways. However, their rich coordination chemistry and redox properties are such that they are capable of escaping out of the control mechanisms such as homeostasis, transport, compartmentalization and binding to the designated tissue and cell constituents. Breakdown of these mechanisms can lead to the metal binding to protein sites other than those tailored for that purpose or displacement of other metals from their natural binding sites.

A growing amount of results provide evidence that toxic and carcinogenic metals capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules [52].

Metals are known to modulate gene expression by interfering with signal transduction pathways that play important roles in cell growth and development [53]. Deregulation of cell growth and differentiation is a typical characteristic of the cancer phenotype. Actions of metals interfere with deregulation of cell proliferation by activating various transcription factors, controlling cell cycle Eprogression and apoptosis [53].

1.6.1. Essential metals

1.6.1.1 Iron (Fe)

Iron occurs in the oxidation states (+2) and (+3). The ferrous ions are soluble in biological fluids and generate in the presence of oxygen damaging hydroxyl radicals. The ferrous ions are unstable in aqueous media [54] and tend to react with molecular oxygen to form ferric ions and superoxide anion radical.

The oxidized form of iron is insoluble in water at neutral pH and precipitates in the form of ferric hydroxide [55]. Paradoxically, despite the fact that both iron ions, ferrous and ferric are so inaccessible, iron is the key catalytic site of many of the enzymes and oxygen-transporting proteins in cells [56].

1.6.1.2. Copper (Cu)

The two common oxidation states of copper(Cu) metal are Cu (II) and Cu (I). The essential trace element copper is a cofactor of many enzymes involved in redox reactions, such as cytochrome oxidase, ascorbate oxidase, or superoxide dismutase. In addition to its enzymatic roles, copper is used in biological systems for electron transport [57]. The blue copper proteins that participate in electron transport include azurin and plastocyanin [58].

1.6.1.3. Zinc (Zn)

Zinc is a known trace element found in plants and animals. The adult human body contains approximately (1.5–2.5) grams of zinc, present in all organs, tissues, fluids and secretions [59]. Zn not only enhances the action of insulin and manages blood glucose concentration, but also plays an essential role in the development and maintenance of the immune system [60]. In contrast, Zn deficiency causes growth retardation and hypogonadism, loss of appetite, dermatitis, reduced taste acuity, delayed wound healing, impaired reproduction, and poor immune function [61]. Zn deficiency is related to poor dietary intake, excessive dietary phytate intake, chronic illness, or over-supplementation of Cu [62].

Zinc plays an essential role in cell membrane integrity, and is a component of more than 300 different enzymes that function in many aspects of cellular metabolism, involving metabolism of proteins, lipids and carbohydrates [63].

The functions of Zn comprise the stabilization of conformation in transcription factors. Zn also modulates cellular signal transduction processes [64].

1.6.1.4. Manganese(Mn)

Manganese (Mn) is the 12th most abundant element and composes approximately 0.1% of the earth's crust. The human population is readily exposed to Mn through soil erosion, resulting in the presence of Mn in food, air and waterways.

In its natural form in the environment, Mn exists usually as oxides, carbonates, and silicates, with Mn dioxide being the most commonly found natural form. Organic Mn is highly present in the environment through common human uses, such as in smoke inhibitors and the antiknock

gasoline additive methylcyclopentadienyl manganese tricarbonyl (MMT). It also has a limited use as a medical magnetic resonance imaging contrast reagent.

Moreover, inorganic forms of Mn can be found in high concentrations in various industrial settings, as it is used in the manufacturing of dry-cell batteries, fireworks, ceramics, glass, leather and textiles.

B. NON-ESSENTIAL METALS

1.6.2.1. Cadmium (Cd)

An environmental source of Cd is the earth's crust. It is often associated with zinc, lead and copper ores and is extracted as a byproduct during the production of zinc, lead and copper. Anthropogenic sources include batteries and to a lesser extent pigments, coatings, stabilizers for plastics and nonferrous alloys, and photovoltaic devices. Sources of cadmium in the food supply include shellfish, leafy vegetables, potatoes and grains, peanuts, soybeans and sunflower seeds as well as tobacco and organ meats. Cd bio accumulates and this represents the primary source of cadmium exposure aside from occupational exposure and tobacco smoke. Occupational exposure is the result of smelting and electroplating, which require heating of Cd-containing materials.

Tobacco smokers experience nearly double the cadmium exposure compared to nonsmokers. Routes of exposure include inhalation and ingestion.

1.6.2.2. Lead (Pb)

Lead (Pb) is an easily molded, corrosion resistant heavy metal found in the earth's crust and has many industrial uses. Pb is used to manufacture pipes, vehicle batteries, ammunition, and radiation protection equipment, it is also found as a pigment in paints and dyes. Pb compounds were used as gasoline additives in the United States until 1996, and are still in use in some developing countries. Pb is naturally released into the air by weathering and erosion, although the majority of environmental Pb results from burning gas, coal, oil and waste, and from release of lead into acidic water and soil from pipes, paints, and waste [65]

Humans are exposed to inorganic Pb through ingestion of contaminated drinking water, soil, and Pb-based paints and through inhalation of Pb containing dust particles which can be significant in many manufacturing and construction occupations [65].

1.6.2.3. Nickel (Ni)

Nickel is a naturally abundant element and has extensive industrial uses. It is emitted from both natural and anthropogenic sources into the atmosphere [66]. It has many adverse effects on humans, and causes allergies, nasal and lung cancer, and kidney and cardiovascular diseases owing to the inhalation of contaminated air [67, 68].

1 .6.2.4. Chromium (Cr)

Chromium is a cancerous and toxic element. In the environment, it exists in two stable oxidation states: chromium (III) and chromium IV). Chromium (III) is a less hazardous form of chromium (VI). They can interconvert to each other during industrial operations. However, conversion of chromium (VI) to chromium (III) is less harmful to the environment because the latter is lower in toxicity.

Chromium is used in many industries that pose a threat to regional climates. In comparison to natural chromium emissions from the environment, ferrochrome industry emissions are at the highest level [68].

CHAPTER TWO

1. Analytical Methodology for analysis of metals in plant leaves

2.1. Statements of the problem

Traditionally, *Thymus schimperi* is widely used in Ethiopia in various forms. The fresh or dried leaves of *Thymus schimperi* are commonly utilized as tea, as well as in the preparation of traditional Ethiopian spices such as "berbere" and "mitten shiro." Additionally, the fresh leaves are often employed in the washing and fumigation of jars used for milking and baking. These practices are particularly common among the people living in the Bale Zone of the Oromia region.

Given the traditional significance of *Thymus schimperi*, the present study was conducted to identify the levels of metals in the leaves of Thymus schimperi collected from different areas within Goba and Fenkel in the Bale Zone. These locations were chosen specifically because they are free from industrial contamination or agricultural pollutants, such as weed killers or artificial fertilizers. The study aims to provide insights into the metal content of Thymus schimperi leaves from areas not impacted by common sources of environmental contamination.

2.2. Objectives of the study

2.2.1. Geneal objectives

The overall objectives of this research is to investigate the levels of selected metals in Tosign leaves cultivated in different areas of Goba and Fenkel (Bale zone).

2.2.2. Specific objectives

To develop and validate sample preparation procedure for the determination of metal.

To determine the levels of metals (Cr, Cu, Pb, Cd, Zn) in Tosign leaves by Flame Atomic Absorption Spectroscopy.

To compare the levels of the selected metals in Ethiopia *Thymus schimperi* with the WHO standard and reported literature.

CHAPTER THREE

3.Experimental

3.1. Instrumentation and Apparatus

A ceramic pestle and mortar were employed to grind the dried Tosign (*Thymus schimperi*), ensuring the material was finely processed. A digital analytical balance was used to accurately weigh the samples, and polyethylene plastic bags were utilized for drying the samples efficiently. For the digestion of samples, a 250mL Quick-fit round bottomed flask, equipped with a reflux condenser, was used in conjunction with a Kjeldahl apparatus hot plate.

Various pipettes and micropipettes were utilized to measure precise volumes of acids and standard solutions, as well as for spiking known concentrations during recovery tests. Volumetric flasks of 25mL and 50mL capacities were used to dilute the sample solutions and prepare standard solutions as needed for the analysis.

The analysis of metals, including zinc (Zn), cadmium (Cd), chromium (Cr), lead (Pb), and copper (Cu), was conducted using a ZEEnit 700p scientific model flame atomic absorption spectrometer (Analytikjena, Germany). This instrument was equipped with a deuterium arc background corrector and operated with an air-acetylene flame to ensure precise and accurate measurement of the metal concentrations.

3.2. Chemicals, Reagents and standard solutions

For the digestion of Tosign leaves samples, 69.72% nitric acid (HNO_3) and 70% perchloric acid ($HClO_4$) were utilized. Stock standard solutions of 1000 mg/L for the metals zinc (Zn), cadmium (Cd), chromium (Cr), lead (Pb), and copper (Cu) were employed. These stock solutions, prepared as nitrates for each element in 2% HNO_3, were used to create calibration curves necessary for the accurate determination of metal concentrations in the samples. Distilled- water was used for the dilution of standard solutions and for rinsing purposes, ensuring the purity and consistency of the experimental conditions. All reagents used in the analysis were of analytical grade, and all standard solutions were certified reference materials, guaranteeing the reliability and accuracy of the measurements.

3.3. Working procedure

3.4. Area of the study

The leaves sample of Tosign were collected from various areas in Ethiopia, specifically from gardens located in Bale Zone of the Oromia region, around Goba Ketema and Goro(Fenke).The selection of these sample collection sites was primarily based on their accessibility for sampling and the high demand for the Tosign plant in these areas. Additionally, these locations were chosen because they are free from industrial waste, far from agricultural activities, and offer a natural environment ideal for accurately identifying the metal content in Tosign leaves.

3.4.1. Geographical description of sample collection site

A. BALE GOBA

Goba (Oromo: Gobbaa, Amharic: ጎባ) is a town and separate woreda located in the Bale Zone of the Oromia Region, Ethiopia approximately 446 km southeast of Addis Ababa, this city has a latitude and longitude of 7°0′N 39°59′E and an elevation of 2,743 meters above sea level. Owing to its very high altitude, Goba has a subtropical highland climate (Köppen *Cwb*) with cool to cold mornings and mild to warm afternoons year-round. There is a lengthy rainy season from February/March to October, and a short dry season with chillier mornings from November to January.

B. Goro(Fenkel)

Goro is a town in southern Ethiopia. Located in the Bale Zone of the Oromia Region, this town has a longitude and latitude of 6°59′N 40°30′E and an elevation of 1650 meters above sea level. It is the administrative center of Goro woreda. This place is situated in Bale, Oromiya, Ethiopia, its geographical coordinates are 6° 59' 0" North, 40° 30' 0" East .

Part of the Bale Zone, Goro is bordered on the southwest by Guradamole, on the west by Berbere, on the northwest by Sinanana Dinsho, on the northeast by Ginir.

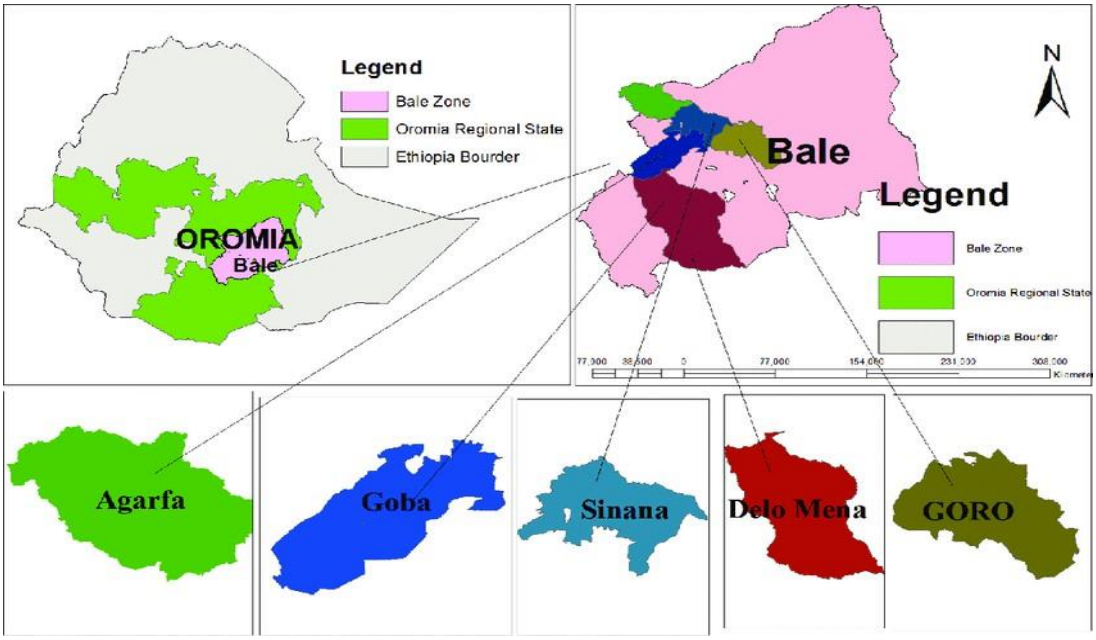

Figure 0.1Map-of-Agarfa-Goba-Sinana-Dello-Menna-and-Goro-distrcts-of-Bale-zone

3.5. Sample collection

The samples were collected from various locations in Bale Goba, specifically around Batu preparatory and secondary School and Fenkel (near Gulbaduma Primary School). The collection was done randomly, and the samples were then mixed together to ensure a representative composite sample. The collected samples were packed in polyethylene plastic bags and transported to the laboratory, where they were prepared for further analysis.

3.6. Apparatus cleaning

To prevent contamination, all necessary materials were washed with detergents and tap water, rinsed with deionized water and dried at room temperature and kept in dust free place until needed for use.

3.7. Sample preparation for elemental analysis

The collected *Tosign* leaves, along with their stems, were thoroughly washed with running tap water and then rinsed with distilled water to remove any impurities. The leaves were initially allowed to air dry for 5 days. After this initial drying period, the leaves were separated from the stems and then further air-dried for an additional 5 days. Following air drying, the leaves were oven-dried at 100°C for 24 hrs to ensure complete moisture removal.

Once dried, the leaves were finely powdered using a mortar and pestle, then sieved to achieve a uniform particle size. The powdered sample was stored in a desiccator to prevent moisture absorption before digestion. For the digestion process, 0.5 g of the powdered sample was taken, and a solution was prepared for the final determination of metal content.

The following figures shows Tosign plant on the land before collected, after collected

Figure 0.2 Tosign plant photo on land in the location of Bale Goba(around batu high school)

Figure 0.3 The collected Tosign sample before separation from the stem (photo)

3.8. Optimization of digestion procedure

Sample preparation is a critical factor in achieving accurate and reliable analytical results. The digestion process plays a key role in this, as it is designed to separate the analyte from its matrix and prevent interactions between organic substances and metal ions or chemical reagents. The choice of an appropriate digestion procedure depends on several factors, including the nature of the organic material, the content of inorganic components, and the specific heavy metal targeted for analysis. The selected digestion method must align with the requirements of the analytical technique used.

Heavy metals in soil or plant matter are often bound to organic materials, necessitating the use of acids to break these bonds and facilitate the extraction of metals. Acid digestion is particularly important for ensuring that the metals become soluble, which is essential for accurate analysis.

To determine the most effective digestion procedure, several parameters were optimized, including digestion time, reagent volume, the ratio of reagents, and digestion temperature. These parameters were adjusted one at a time while keeping the others constant to identify the

conditions that produced a clear solution at lower temperatures, required minimal reagent volume, and reduced digestion time.

Ultimately, the optimal digestion procedure for Tosign was determined based on these criteria. The chosen method required 3 hrs for the complete digestion of a 0.5 g sample using 3 mL of 69.72% HNO_3 and 1.0 mL of 70% $HClO_4$, as outlined in Table 1. This approach provided a reliable and efficient digestion process, ensuring accurate determination of the metal content in the samples.

Table 0.1 Different procedures tested during optimization of Thyme leaf sample digestion

No	Amount of Tosign(g)	Volume of reagent(mL)	Digestion Temperature(^{0}C)	Digestion time(hrs)	Results after filitration and dilution
1	0.5	HNO_3:$HClO_4$, 2:1	240	3	Clear but cloudy
2	0.5	HNO_3:$HClO_4$, 3:1	240	3	Clear solution
3	0.5	HNO_3:$HClO_4$, 4:1	240	3	Light yellow
4	0.5	HNO_3:$HClO_4$, 3:1	210	3	Clear solution
5	0.5	HNO_3:$HClO_4$, 3:1	240	3	Clear solution
6	0.5	HNO_3:$HClO_4$, 3:1	270	3	Cloudy solution
7	0.5	HNO_3:$HClO_4$, 3:1	210	3	Clear solution
8	0.5	HNO_3:$HClO_4$, 3:1	210	2:00	Cloudy solution
9	0.5	HNO_3:$HClO_4$, 3:1	210	2:30	Clear but slight yellow
10		HNO_3:$HClO_4$, 3:1	210	3	Clear solution(optimum)

3.9. Digestion of Tosign Leaves

Using the optimized procedure, 0.5 g of powdered Tosign sample was accurately weighed on an analytical balance and placed in a 250 mL round bottomed flask. To this, 3 mL of 69-72% HNO_3 and 1mL of 70% $HClO_4$ were added. The round bottomed flask was then fitted with a reflux condenser and heated on a Kjeldahl apparatus on a hot plate for three hrs at a temperature of 210°C. After the heating process, the digest was allowed to cool for 15 min without dismantling the condenser. It was then further cooled to room temperature for an additional 10 min after dismantling the condenser.

Following cooling, the mixture was diluted with 10 mL of distilled water and filtered through Whatman filter paper into a 50 mL volumetric flask. The round bottom flask was further rinsed with 5 mL of distilled-deionized water, and the rinse was added to the filtrate. The flask was then filled to the mark with distiled water to achieve the final volume.

The digestion process was performed in triplicate to ensure accuracy and repeatability. Additionally, triplicate blank samples, consisting of 3 mL of HNO_3 and 1 mL of $HClO_4$, were digested following the same procedure as the sample to serve as controls.

All the digests, including the sample and blanks, were stored properly until they were analyzed using Flame Atomic Absorption Spectroscopy (FAAS). This meticulous process ensured the reliability of the analytical results.

3.10. Determination of levelsof selected metals by FAAS

Trace elements in plants play a significant role in the effectiveness of medicinal preparations. The elemental composition of various plant parts, including leaves, seeds, and fruits, is often determined using Flame Atomic Absorption Spectrophotometry (FAAS).

Flame Atomic Absorption Spectrophotometry (FAAS) is a widely used analytical technique for measuring the concentration of elements in a sample. This technique relies on the absorption of light by atoms in the sample. When atoms absorb ultraviolet or visible light, they transition to

higher electronic energy levels. The concentration of the analyte is determined by measuring the amount of light absorbed by the sample.

Key points about AAS include:

- Concentration measurements are typically derived from a calibration curve, which is established by calibrating the instrument with standards of known concentration.
- AAS is a common and reliable technique for detecting metals and metalloids in environmental and biological samples.

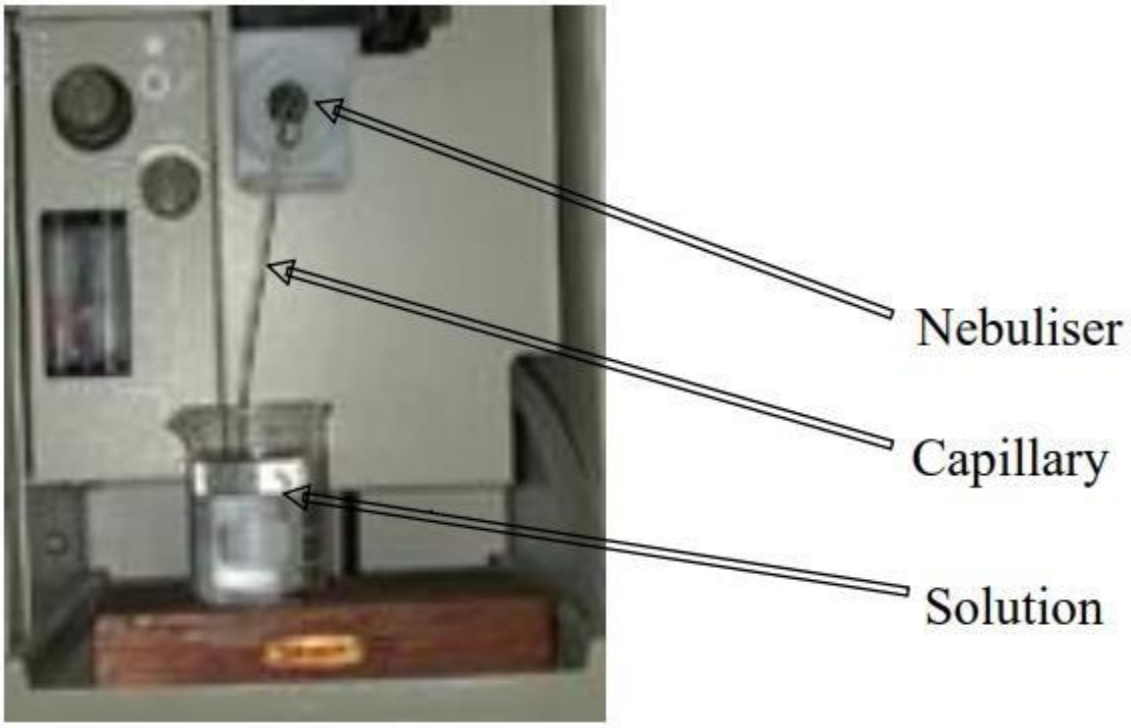

Figure 0-4 Atomic Absorption Spectrometer sample introduction system

3.11. Calibration of the instrument

A 10 mg/L intermediate standard solution was prepared in a 100 mL volumetric flask by diluting a 1000 mg/L stock solution used for Flame Atomic Absorption Spectroscopy (FAAS). From this 10 mg/L solution, four working standard solutions were prepared at different concentrations for each metal (Zn, Cd, Cr, Pb, and Cu).

The instrument was calibrated using these working standards after optimizing the parameters, such as energy, slit width, lamp current, and wavelength, to achieve maximum signal intensity. Following calibration, all Tosign samples and blank samples were analyzed for each metal using the absorption mode of FAAS to determine their concentrations accurately.

Table 0.2 Instrumental operating conditions for the determination of metals using FAAS inThyme Schemperi

Metal	Wavelegth(nm)	Method detection limit(mg/l)	PMT	Slit width(nm)	Lamp current(mA)
Zn	213	0.1522	391	0.5	2
Cd	228	0.0791	253	1.2	2
Cr	357	0.008	203	0.2	4
Pb	283	0.007	233	1.2	2
Cu	324	0.027	243	1.2	2

Table 0.3 Working Standard solutions, absorbance, equation of the calibration curves and correlation coefficient for determination of metals using FAAS

No	Metal	Working standard concentration(mg/L	Absorbance	Equation of the calibration curve	Correlation coefficient($R^{2)}$	
.1	Zn	0	-0.0064	y=0.54592x+0.008	0.9971	
		0.25	0.15951			
		0.5	0.27046			
		0.75	o.42746			
		1	0.54778			
2	Cd	0	0.00003	y=0.0537 x -0.0501	0.9958	
		0.25	0.05685			
		0.5	0.12069			
		0.75	0.26159			
		1	0.21630			

3	Cr	0	-0.00309	y=0.08641x-0.0006	0.9963	
		0.25	0.02295			
		0.5	0.04393			
		0.75	0.06536			
		1	0.08372			
4	Pb	0	0.0682	y=0.03272x+0.009	0.9993	
		0.25	0.01478			
		0.5	0.02382			
		0.75	0.03119			
		1	0.03952			
5	Cu	0	0.00009	=007223x-0015	0.9993	
		0.25	0.15951			
		0.5	0.27046			
		0.75	0.42746			
		1	0.54778			

1.12. Calibration curves of standard solutions of metals

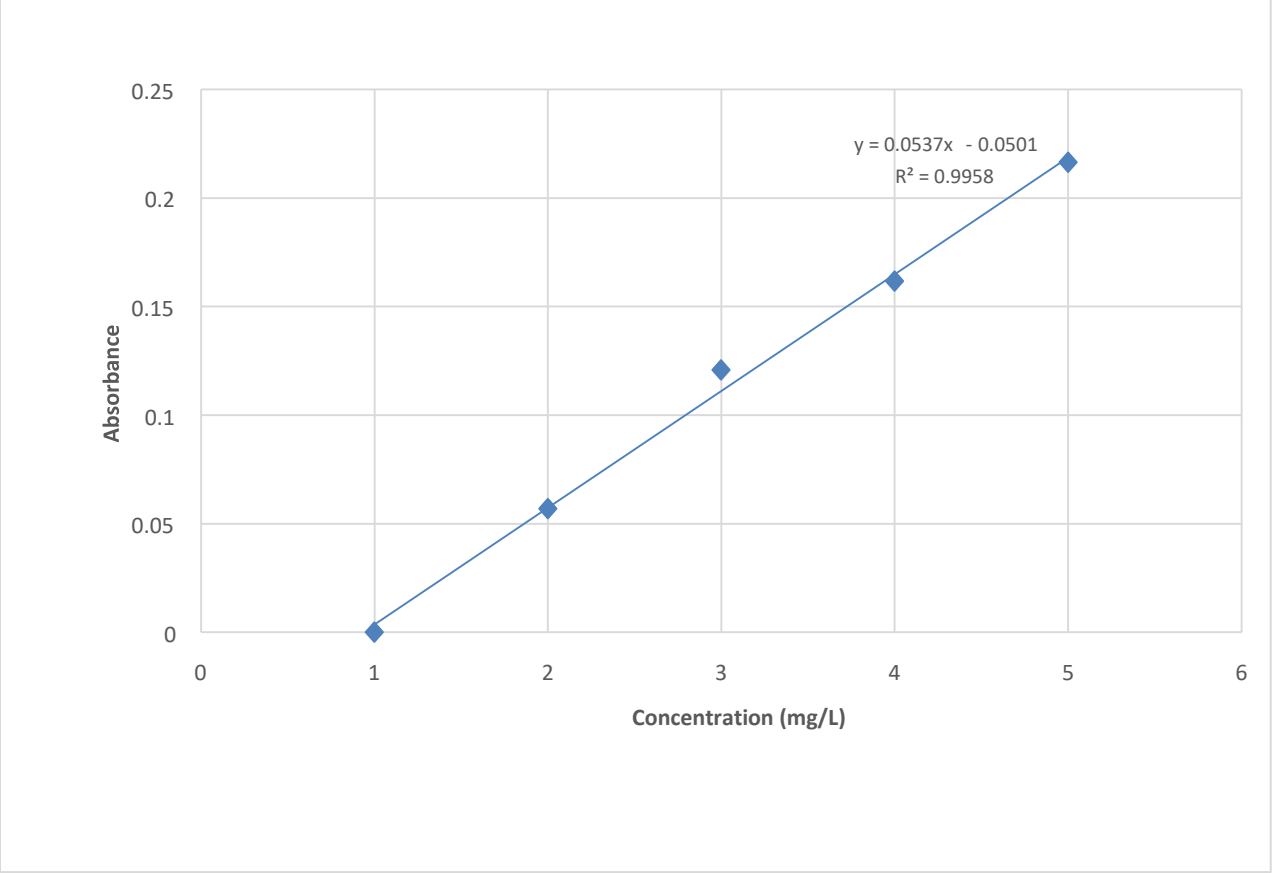

Figure 0.5 Calibration curve for cadmium

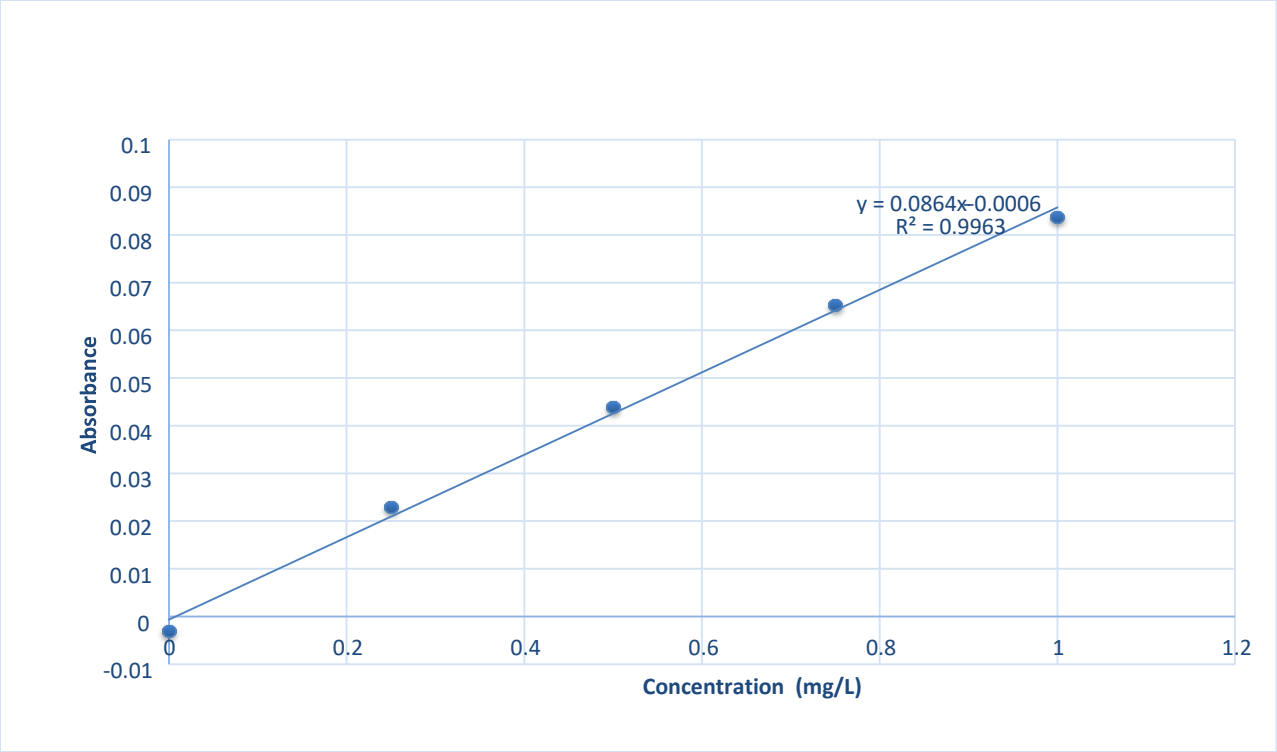

Figure 0.6 Calibration curve for Chromium

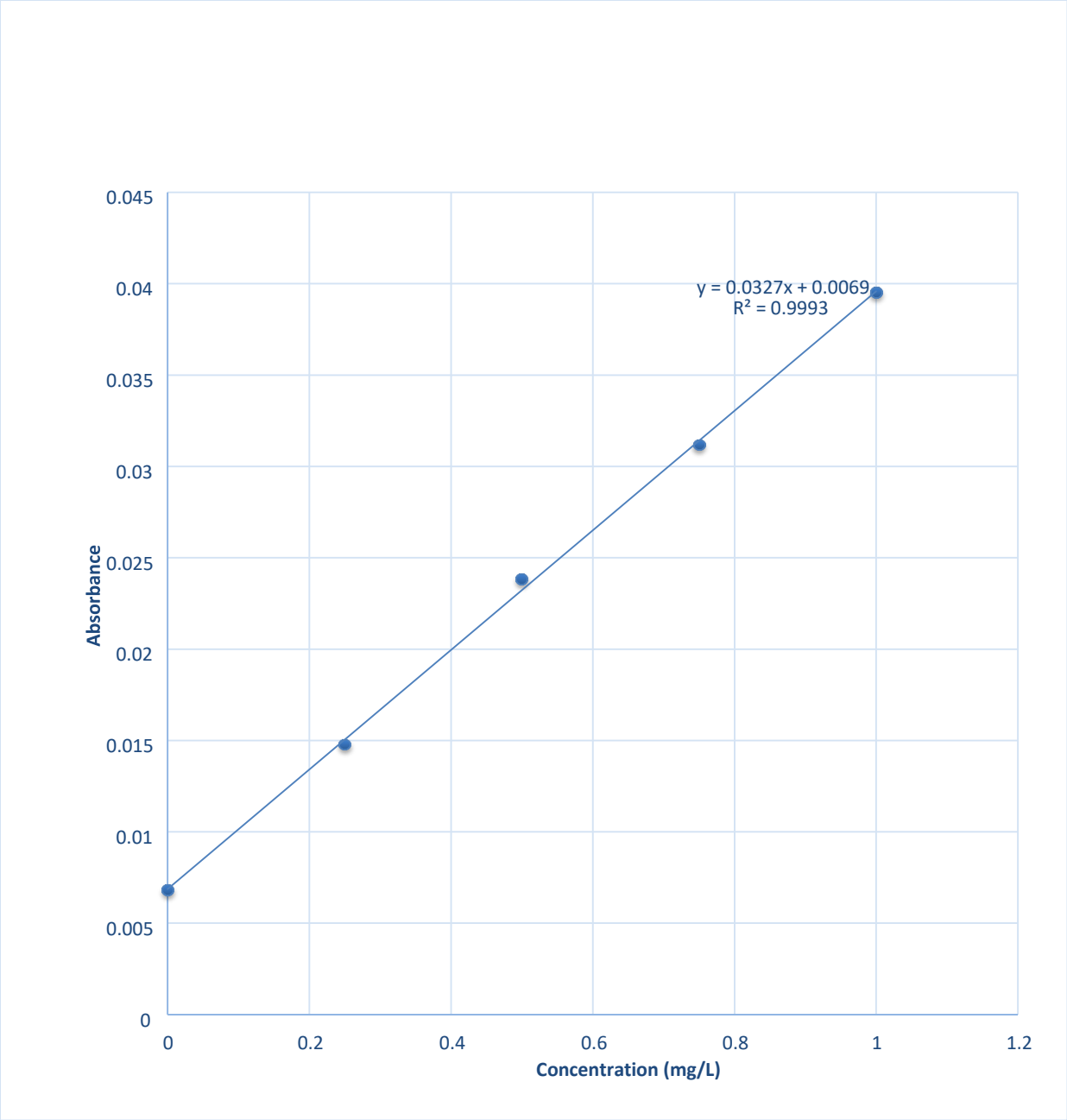

Figure 0.7 Calibration curve for Lead

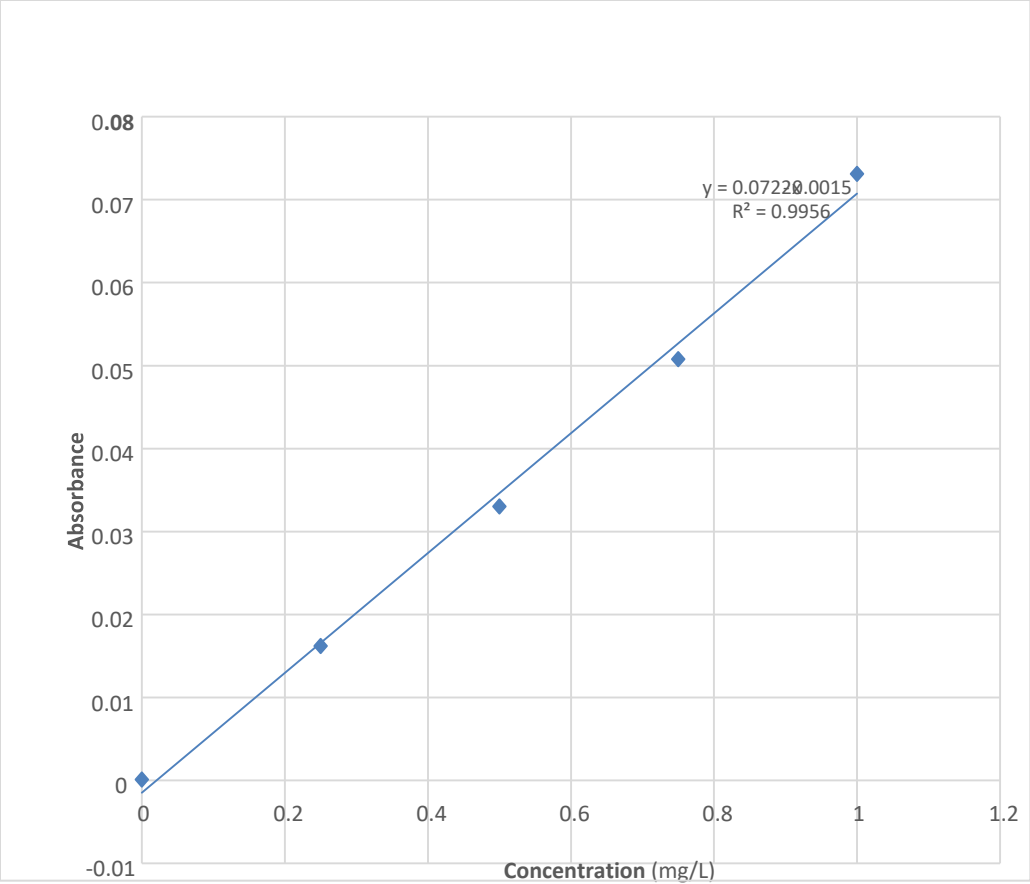

Figure 0.8 Calibration curve for Copper

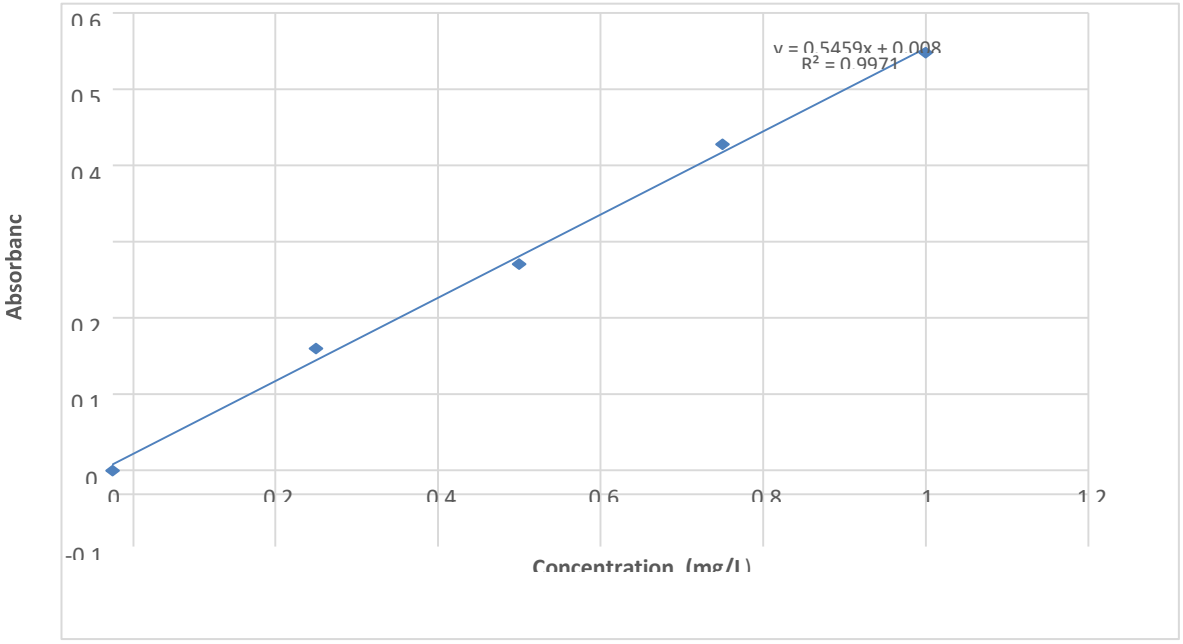

Figure 3.9 Calibration curve for Zinc

The concentrations of metals (Zn, Cd, Cr, Cu, Pb) were determined using Flame Atomic Absorption Spectroscopy (FAAS) after optimizing the instrument's operating conditions to achieve maximum signal intensity. Triplicate determinations were performed on the Tosign samples to ensure accuracy and reliability.

For each metal, a hollow cathode lamp was used, operating under the manufacturer's recommended conditions, corresponding to the primary source line for each element. The acetylene and air flow rates were carefully adjusted to maintain optimal flame conditions, ensuring precise measurement of metal concentrations.

The same analytical procedure was applied to the determination of elements in the digested blank solutions, serving as controls to validate the accuracy of the measurements.

3.13 Precision and Accuracy

A. Precision: is the ability to produce the same or close to the same value multiple times. If someone was shooting at a target, their aim would be precise if all of their shots were at or near the exact same point. High precision coincides with low sample standard deviation. Standard deviation is a measurement of how widely spread a data set is. A sample standard deviation is specifically the spread of data of a particular sample and thus may not represent the true spread of data of the population.

To ensure the precision of the analysis, each sample was digested in triplicate and triplicate reading was carried out [69]. The precision of the results was evaluated by the relative standard deviation of each metal analyze for each sample. % RSD is calculated as follows:

$$\% \text{ RSD} = \frac{SD}{mean} \times 100\%$$

Where: SD = standard deviation of the results

Table 0.4 Relative standard deviation of the analyzed metals

Metal	Cd	Cr	Cu	Pb	Zn
RSD (%)	14.8	-	1.28	2.47	5.94

Using the above formula for measurements of Cu, and Pb % RSD were found in the acceptable range (% RSD < 5) .These showed that the result was highly precise but for measurements of Cd and Zn %RSD were > 5 these showed that the result was less precise**.**

B. Accuracy: is the degree to which the result of a measurement conforms to the correct value or a standard and essentially refers how close a measurement is to its agreed value. A measured value that's far from a true value is inaccurate, while a measure that is close to a true value is accurate. High levels of accuracy are needed to correctly identify a population's value. A sample with an inaccurate mean will not correctly represent a trend seen within a population

CHAPTER FOUR

2.Results and Discussion

4.1. Method Detection Limit

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be reliably reported with 99% confidence that the measured concentration is distinguishable from that of a method blank. Method blank samples are used to calculate this confidence level, ensuring that the results are attributed to the sample rather than contamination.

The detection limit of an analytical procedure represents the smallest amount of analyte in a sample that can be detected with confidence. It is typically determined using methods such as the signal-to-noise ratio, the standard deviation of the response, and the slope of the calibration curve. The signal-to-noise approach is applicable to analytical procedures that exhibit baseline noise.

To determine the detection limit, measured signals from samples with known analyte concentrations are compared with those from blank samples. The minimum concentration at which the analyte can be reliably detected is established through this comparison.

The method detection limit (MDL) can be mathematically expressed as:

MDL=3SD where SD the standard deviation of the responses is the slope of the calibration curve.

Table 0.1 Method detection limit of metals

Metals	Cd	Cr	Cu	Pb	Zn
MDL(mg/l	0.007	0.734	0.093	0.168	0.167

4.2. Recovery Test

The recovery studies are clearly an essential component of the validation and use of all analytical methods. It is important that all concentrated with the product on and interpretation of analytical result are aware of the problems and the basis on which the result being reported. The mean

percent recovery of any sample type should meet design specifications which are typically 80-120%.

Percentage recovery of spiked sample can be calculated using the following method.

$$\%\text{Recovery} = \frac{\text{spiked sampleconcentration} - \text{unspiked sample concentraion}}{\text{known spiked sample added}} \times 100$$

Table 0.2 Recovery results for the analyzed Tosign leaves

Metal	Unspiked sample(mg/L)	Spiked amount(mg/L)	Concentration of metal in spiked sample (mg/L)	% recovery
Cd	0.018	0.00724	0.0249	93.16
Cu	0.188	0.07536	0.254	87.13
Pb	0.375	0.15808	0.558	103
Zn	0.853	0.3412	1.194	99.82
Cr	BDL			

The percentage recoveries of metals given above in the table 5.These values were within the acceptable range of 80-120% expected. So FAAS method was a good accuracy of analytical procedure.

4.3. Levels of Metals in Tosign leaves

The developed FAAS method was utilized to determine the concentrations of metals (Cd, Cr, Cu, Pb, and Zn) in the leaves of Tosign collected from various locations in Bale Goba and Fenkel. The mean values were calculated based on triplicate analyses of the samples. The accuracy and precision of the results were validated using different statistical methods after determining the metal levels in the Tosign leaf samples. The results, expressed in mg/L on a dry weight basis, along with the corresponding standard deviation values, are presented in Table 6.

Table 0.3 Mean concentration and standard deviation in mg of selected metals in the sample analyzed by FAAS

NO.	Metal	Mean concentration(mg/L)
1	Cd	0.018± 0.003
2	Cr	BDL
3	Cu	0.188 ± 0.002
4	Pb	0.375± 0.009
5	Zn	0.853 ± 0.051

4.4. Comparison of Metals in leaves Tosign leaves

Metals collected from the different sources are released in to the environment tend to bio accumulate in plants, organisms and even biomagnified in the food chain were human beings are highly exposed. So, if Tosign leaves contained with heavy metals, metals will be Trans feed to animals and humans immediately and it affects human health if the concentration of heavy metals are above threshold limit.

The concentration of the selected metals (Cd, Cr, Cu. Pd and Zn) obtained in this study were Cd 0.018 ± 0.003, Cr BDL, Cu 0.188 ± 0.002, Pb 0.375 ± 0.009 and. Zn 0.853 ± 0.051.The concentration of heavy metals in leave Tosegn sample vary in the order of Zn >Pd >Cu >Cd, but the concentration of Cr is below the detected limit.

Bar graph for metas in Tosign samples

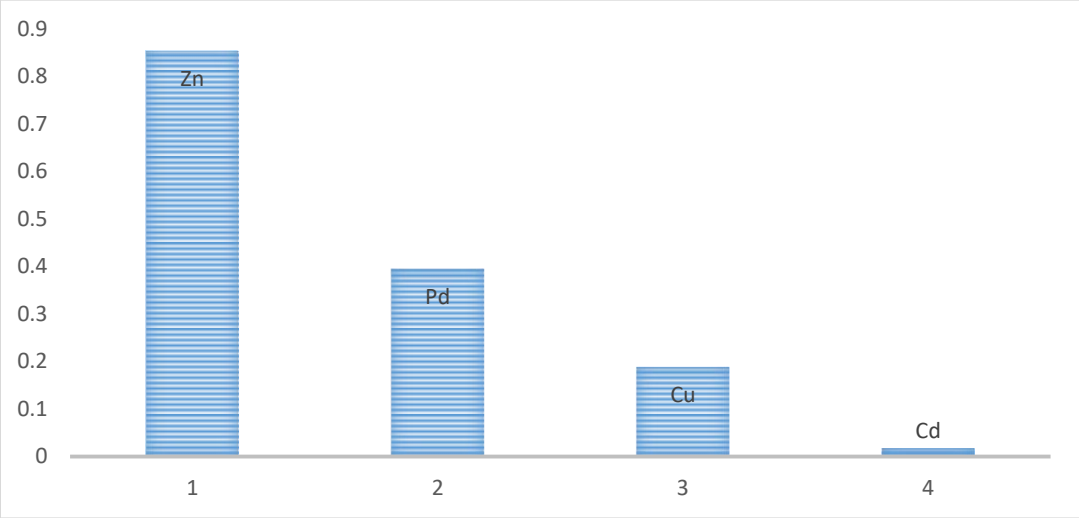

Figure 0.1 Concentration of the selected heavy metals (Cd,Cu,Pd and Zn)

Metals released into the environment from various sources tend to bioaccumulate in plants and organisms, and can even biomagnify through the food chain, leading to significant exposure for humans. If *Tosign* leaves contain heavy metals, these metals can be transferred to animals and humans, potentially impacting human health if their concentrations exceed threshold limits.

In this study, the concentration of metals detected in *Tosign* leaves was generally lower than the maximum allowable concentrations set by the World Health Organization (WHO).

The concentrations of the selected metals (Cd, Cr, Cu, Pb, and Zn) detected in this study were as follows (all values in mg/kg on a dry weight basis):

- Cadmium (Cd): 0.018 ± 0.003
- Chromium (Cr): Below detectable limit (BDL)
- Copper (Cu): 0.188 ± 0.002
- Lead (Pb): 0.375 ± 0.009
- Zinc (Zn): 0.853 ± 0.051

The concentration of heavy metals in *Tosign* leaves followed the order: Zn > Pb > Cu > Cd. However, the concentration of chromium (Cr) was below the detectable limit. These findings

highlight the importance of monitoring heavy metal levels in plants used for consumption, as elevated levels, particularly of zinc in this case, could pose health risks if consumed in large quantities.

4.5. Comparison of Metals in Tosign Leaves obtained in the present study with those Reported in the Literature and WHO permissible values

The concentration of the selected heavy metals (Cr, Cd. Cu, Pb and Zn) in Tosign leaves has been reported in literatures however with variations in effect of fertilizers, water, or irrigation used by farmers.

Table 0.4 Comparison of selected metal concentrations in Tosign leaves sample of this study with those reported in the literature and WHO report

Metal	Ethiopia (mg/L)	Debresina(Ethiopia	Eropean(vellore)mg/kg	Egypt (mg/L)	WHO (mg/kg)
Cd	0.018 ±0.003	1.3±0.08	0.00-0.51	2.44	0.0116-0.306
Cr	ND	NR	0,23-2,08	33.75	NR
Cu	0.188± 0.002	8.9±0.56	1.28-13.47	11.40	11.55-34.19
Pb	0.375± 0.009	NR	1,71-9,01	14.4	0.024-0.387
Zn	O.8525± 0.051	NR	5.08-23.47	68.80	17.94-187.24
Reference	This study	[28]	[79]	[70]	[71]

Key: ND-not detected and NR-not reported

CHAPTER-FIVE

2. Conclusion and Recommendations

5.1 Conclusion

The mean concentrations of metals (Cd, Cu, Pb, and Zn) in the analyzed *Tosign* leaves from this study were found to be lower than those reported in literature from Egypt [70] and were below the maximum allowable concentrations set by the World Health Organization (WHO) [71], making them safe for human consumption.

For this study, the Tosign leaves samples were collected from Goba, specifically around Batu High School and Fenkel, areas that are far from roads, agricultural zones, and industrial activities. The concentrations of metals determined in these samples were very low compared to the WHO's allowable limits, further confirming the safety of these leaves for consumption.

5.2. Recommendations

This research was conducted by collecting samples from small, specific areas that were free from contamination by various human activities. However, there are regions in Ethiopia where Tosign grows in contaminated environments and is traditionally used by humans.

To address the limitations of this study, it is recommended that future researchers take more time and collect a larger number of samples from diverse locations, including those areas with potential contamination. This broader approach would provide a more comprehensive understanding of the metal content in Tosign across different environmental conditions in Ethiopia.

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