

Madda Walabu University
College of Natural and Computational Sciences
Department of Chemistry



**Evaluation of Essential and Nonessential Metals in Selected
Indigenous Fruits in Dodola Woreda, Ethiopia**

Thesis

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October, 2024
Bale Robe, Ethiopia

Madda Walabu University

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Indigenous Fruits in Dodola Woreda, Ethiopia**

**A thesis submitted to Department of Chemistry in Partial Fulfillment of
the Requirements for the Degree of Master of Science in Chemistry
(Analytical Chemistry)**

By: Ziyad Geleto

Advisor: Fekadu Roge (PhD)

October, 2024

Bale Robe, Ethiopia

Declaration

This is to certify that the thesis prepared by **Ziyad Geleto** entitled on “**Evaluation of Essential and Nonessentials Metals in Selected Indigenous Fruits in Dodola Woreda, Ethiopia**” is submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science compiles with the regulations of the university and meets the accepted standards with respect to originality and quality.

Name of Student

Signature

Date

Approved By:

Fekadu Roge (PhD)

Advisor

Signature

Date

Dedication

This study dedicated to my family!!

Approval Sheet

As Thesis advisor, I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by **Ziyad Geleto** entitled: **Evaluation of Essential and Nonessentials Metals in Selected Indigenous Fruits in Dodola Woreda, Ethiopia**. Therefore I recommend that it can be submitted as for defense since it fulfills all the requirements.

Fekadu Roge (PhD) _____

Advisor

Signature

Date

As member of the Board of Examiners of the MScthesi open defense examination, we certify that and evaluated the thesis prepared by **Ziyad Geleto** and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirements for the Degree of Master of **Science in Analytical Chemistry**.

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Biographical Sketch

The author was born in 1986 E.C. in Dodola Woreda, Oromia Region, Ethiopia. He attended his primary school in Dodola Junior School (1-8) in 1994 to 2001 E.C and his Secondary school in Dodola Secondary School (9 to 12) in 2002 to 2005 E.C. Then the author was joined Mizen Tepi University in 2006 E.C to attended regular program and graduated in Bachelor Degree of Science in Chemistry in academic year of 2009 E.C. He has been teaching chemistry at primary and secondary schools from 2010 to 2016 E.C., totaling 7 years. Then in year? he joined Madda Walabu University for his Master of Science Degree in Chemistry. His research area focused on the **“Evaluation of Essential and Nonessential Metals in Selected Indigenous Fruits in Dodola Woreda, Ethiopia”**.

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Arconomy and Abbreviations

AAS	Atomic absorption spectrometry
EC	Electrical Conductivity
LOD	Limit of Detection
LOQ	Limit of quantification
pH	Power of hydrogen
TDS	Total dissolved solids

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Abstract

Fruits are a good source of essential and nonessential metals, yet they are less consumed. In Dodola woreda various types of fruits either planted or growing wildly. Indigenous fruits are well distributed among the rural areas and members of the community lack awareness of the nutritional importance of such fruits as Rosa abyssinica and Dovyalis abyssinica fruits and therefore they do not consume well. These indigenous fruits are rich in essential and nonessential elements that help in immune boosting, destruction of free radicals and general physical growth. The main objective of the study was to evaluate essential and nonessential metals of Rosa abyssinica and Dovyalis abyssinica fruits. After sample preparation, and digestion the levels of those metals in both fruits were determined by atomic absorption spectrometry (AAS). The experimental results of each fruit, the mean concentration of essential and non-essential metals (mg/kg) in Rosa abyssinica sample exhibited decreased trend in the order of $Fe (232.8 \pm 0.8) > Zn (84.5 \pm 1) > Mn (4.87 \pm 0.02)$, $Cu (56.4 \pm 0.1)$, $Co (0.1 \pm 0.006)$, $Cd, (0.027 \pm 0.006) > Pb (0.07 \pm 0.006)$, while mean concentration for the Dovyalis abyssinica samples follow the decreasing order of $Fe (273 \pm 1) > Zn (86.3 \pm 0.6) > Mn (4.92 \pm 0.01)$, $Cu (63.6 \pm 0.1)$, $Co (0.1 \pm 0.001)$, $Cd, (ND)$, $Pb (ND)$ slightly a significant difference was observed between the mean concentrations of all metals in Rosa abyssinica and Dovyalis abyssinica fruit. This indicates that the sample contain the higher level of some selected essential metals than non-essential metals in both plants, and in the precision test, %RSD (relative standard deviation) for selected essential and non-essential metals was below the recommended level. Therefore, the content of essential and non-essential metals in both indigenous plants were below the permissible value of WHO/FAO.

Keywords: Indigenous fruit, essential and nonessential metals, Atomic Absorption Spectrophotometric, and Dodola woreda.

Chapter One

1. Introduction

1.1. Background of the study

Plants are the main sources for animal and human nutrition. Depending on different climates and cultures, a number of plant species have been used for different purposes. From past to present, a number of indigenous fruits are being used as food sources (Zelalem *et al.*, 2021). The indigenous fruits may be seasonal and can have more nutritional value than those grown in farmlands (Zelalem *et al.*, 2021). These are fresh and more tasteful. Indigenous fruits are rich sources of various vitamins, minerals, fibers, antioxidants, etc. additionally reduces the risk of several diseases like diabetes, cancer, coronary heart disease, neurodegenerative ailment and aging as well. Ethiopia is endowed with varieties of indigenous fruits which are consumed for their essential and nonessential micronutrient (Zemenay *et al.*, 2021). These indigenous fruits have been shown to possess valuable nutritional properties and therapeutic values. Consumption of dietary antioxidants from these fruits such as *Rosa abyssinica* and *Dovyalis abyssinica* (A. Rich) warb are beneficial in preventing diseases as well as promoting food insecurity in Ethiopia (Mulugeta *et al.*, 2022).

Wild fruits like *Rosa abyssinica* and *Dovyalis abyssinica* contain high nutritional and nutraceutical values, being rich in organic/inorganic sources such as carbohydrates, proteins, lipids, vitamins, essential and nonessential micronutrients (Fe, Mn, Co, Cr, Ni, Cd, Pd) and polyphenols (Natol *et al.*, 2023). *Rosa abyssinica* (Kega) is the family of *Rosaceae* and used as traditional diet and *Rosa abyssinica* local named as Afan Oromo “qaqawii” or Amharic “Kega”. *Dovyalis abyssinica* is the family of *Flacourtiaceae* and local languages of *D. abyssinica* Koshim (Amharic), Ankakute (Afan Oromo) and collected and eaten raw, mostly by children who enjoy the pleasant sweet sour flavour of the fruits. Adults also consume them, but mostly in periods of food shortage (Natol *et al.*, 2023).

Living organisms require varying amounts of “heavy metals” including iron, copper, manganese and zinc are essential micronutrients and required by humans as they play important roles in biological systems, though excessive levels may result in damaging effects to the organism. Other heavy metals such as cadmium and lead are toxic and non-essential micronutrients and

their accumulation over time in human bodies could cause serious illnesses (Sibiyaa *et al.*, 2020). Accurate and adequate food composition data are invaluable for estimating the adequacy of intakes of essential metals and assessing exposure risks from intake of toxic non-essential metals (Zelalem *et al.*, 2021a). For example, the bioavailability of iron in mushrooms is considerably high and human body can absorb up to 90 % of the available form (Gebrelibanos *et al.*, 2016). The recommended dietary allowances (RDA) are 0.9, 8.0-18.0, 8.0-11.0 and 1.8-2.3 mg per day for Cu, Fe, Zn and Mn, respectively. This level is sufficient to meet the nutrient requirement of nearly all (97 to 98 %) healthy individuals, in a particular life stage and gender group (Sibiyaa *et al.*, 2020). Heavy metals contents in different plants, which are used as food source, have been conducted in the world including in Ethiopia. Recently some studies have been done on the mineral contents of fruits of cactus pear, fennel, banana, grape, guava, mandarin and orange, apple and seeds of korarima and fenugreek cultivated in Ethiopia (Shambel *et al.*, 2016; Demisew and Chandravanshi, 2021; Mebrahtu and Chandravanshi, 2016).

1.2. Statement of the Problem

Rosa abyssinica and *Dovyalis abyssinica* are common wild edible fruits. In Ethiopia, wild edible fruits gathered from the wild contribute essential roles in the community's nutrition, mainly to the rural population. Due to a lack of understanding about the nutritional value and health-promoting bioactive chemicals of wild fruits, ignorance and negligence may jointly be blamed for the poor intake of these fruits in some regions (Omari *et al.*, 2017). The fruits of *R. abyssinica* and *D. abyssinica* contain higher quantities of essential micronutrients and lower levels of non-essential micronutrients in their edible parts, depending on soil metal availability and climatic conditions. Since both fruits are consumed without undergoing any process, they pose no risks to consumer's body at all. Hence, the determination of the level of the essential and toxic metals in the wild edible fruits was very important. Despite this, research on wild edible plant fruits has not received significant attention in Ethiopia. Therefore, the proposed study was focused on the evaluation of the essential and nonessential metals accumulated in both fruits.

1.3. Significance of the Study

The study will provide useful information on the levels of essential and nonessential metals in two indigenous fruits in Dodola woreda. This was important to policy makers, agronomists, food and

nutrition security planners, and health workers, NGOs and other organizations interested in food security and promotion of good health. This was a long way in reducing or alleviating poverty, hunger and child mortality especially among the poor members of the community. The study also contributes to the field of knowledge a rich, cheap and affordable micro-nutrient food sources for people and this forms basis for future research in the area. The study helps to promote the propagation of indigenous fruits which enables to improve the economic status of the community and preserve the plants from extinction.

1.4. Objective of the Study

1.4.1. General Objective

- ✓ To investigate the essential and nonessentials metals in the selected indigenous fruits in Dodola woreda.

1.4.2. Specific Objective

- ✓ To determine the level of essentials [Zn, Fe, Cu, Mn, Co] and nonessentials [Cd, Pb] metals of *R. abyssinica* and *D. abyssinica* fruit using atomic absorption spectrophotometer methods.
- ✓ To compare the level of essentials [Zn, Fe, Cu, Mn, Co] and nonessentials [Cd, Pb] metals of *R. abyssinica* and *D. abyssinica* fruit with international standard.

1.5. Research Questions

The research will try to address the following significant questions

1. What are the level of essentials (Zn, Fe, Cu, Mn, Co) and nonessentials (Cd, Pb) metals of *R. abyssinica* and *D. abyssinica* fruit?
- 1.2. How does the level of essential and non-essential metals of *R. abyssinica* and *D. abyssinica* fruit will be compared with that of international standard?

1.2.Scope of the Study

This study focused only on levels of selected essential and nonessential metals in *R. abyssinica* and *D. abyssinica* fruits in Dodola woreda. Only two indigenous fruits were considered due to financial shortage and time constraints.

Chapter Two

2. Literature Review

2.2.Indigenous Fruits in Ethiopia

Wild edible plants are valuable resources forming local survival strategies in food-insecure areas in Ethiopia and for improving the food and nutritional security of households (Alebel and Mohammed, 2021). Rural communities have a strong social and economic attachment to indigenous fruit trees. It is shown that wild edible foods are integral parts of the feeding habits of many communities in most parts of Ethiopia (Alebel and Mohammed, 2021). The consumption of wild edibles is more common in food-insecure areas than in other areas in the country (Teklehaymanot and Giday, 2010). However, food-secure households also regularly enjoy wild edible fruits and plants at some time of the year. It could be surprising that the only sources of nutrition in remote rural communities are wild edible fruits where the domestic fruits are absent in their farming systems and the local markets (Teklehaymanot and Giday, 2010). The community consumes wild edible plants for supplementing staple food, filling seasonal food shortages, and emergency food during a famine in Ethiopia. Those species are primarily utilized for household consumption and are a potential food source in the future. However, much of the research studies on wild edible fruit and plants misunderstood their role in many communities.

Many researchers wrongly concluded that the utilization of wild edible fruits is food insecure for poor people and children. The reality is that food secure households and adults regularly enjoy wild edible fruits and plants. Many marketable wild edible fruits have also made considerable contributions to income generation (Teklehaymanot and Giday, 2010). Several wild edible fruits are on the local markets and are an important source of income. However, the available information on the contribution of indigenous edible fruit species for both household consumption and annual income is very scarce in Ethiopia (Sardeshpande and Shackleton, 2019). Past studies have not gone beyond simply listing the species, and this indicates research gaps on

practical aspects for the development of the species. The other important aspect of many wild edible tree species is their multipurpose utilization. Many wild edible fruit species are widely used and valued for traditional medicine in many countries (Sardeshpande and Shackleton, 2019). This multipurpose nature of the species could be a plausible rationale to further motivate the domestication and wider cultivation of the species.

2.3.Importance of Micronutrients

Micronutrients are nutrients (dominantly vitamins and minerals) required by humans only in small amounts throughout life in order to carry out a vast range of physiological functions (Graham *et al.*, 2007). Micronutrients do not provide energy but help regulate the production of energy from macronutrients. Graham *et al.*, (2007) argues that at least fifty one different nutrients are needed in adequate amounts by human beings. There are nineteen essential micronutrients for physical and mental development, immune system functioning and various metabolic processes (Kennedy *et al.*, 2007).

Two billion people suffer from one or more micronutrient deficiencies demonstrating that hidden hunger is responsible in part for global malnutrition burden (Graham *et al.*, 2007). This is quite evident in the health sector which has been faced with a number of challenges including communicable and infectious diseases, nutrition deficiency disorders and parasitic infections. Adequate intake of micronutrients can be sufficient enough to provide preventive measures either wholly or partially to these healthcare problems. Productivity of the human capacity depends on good health and nutrition. These are iron, zinc, chromium, manganese and copper as trace elements and vitamins A (β -carotene), C (Lascorbic acid), and E (α -tocopherol). Trace element is an element in a sample that has an average concentration of less than 100 ppm. They function in hormones and enzymes through complex series of chemical reactions in the body and are essential in the regulatory and maintenance of normal body functions. These body functions include growth, cells replacement, energy production, reproductive functions, muscles formation and contraction and transmission of nerve impulses (WHO, 1996).

2.4.*Rosa abyssinica* and *Dovyalis abyssinica*

Rosa abyssinica is a prickly evergreen shrub, creeping or often climbing, capable of forming a small tree up to 23 feet (7.0 meters) tall. It has a few prickles on the stem, slightly curved from a

wide base and all similar. It has many variable features. The leaves are compound and leathery. It has 3 pair's leaflets plus one at the tip, each narrowly ovate from 5 to 2 inches (1.3 to 5.1 cm) tip sharp, edge toothed, on a short stalk which is winged by the leafy stipules. Flowers are of fragrant white-pale yellow, and are usually 3 to 20 in dense heads, each stalked, the sepals long, narrow and hairy, soon fall, and have 5 petals about 2 cm long, tip rounded to square (Afshin *et al.*, 2019), with many stamens (Figure 1). The fruits are green at first, but later ripen to orange-red. They are about 1 inch (2.5 cm) long, fleshy and edible with seed within.

Dovyalis abyssinica is a spiny shrub or tree found mostly in Ethiopia and Malawi. It is often found in the upland rain forests, dry evergreen forests, on riverbanks and sometimes in more open woodlands *Dovyalis abyssinica* fruits are collected and eaten raw, mostly by children who enjoy the pleasant sweet sour flavour of the fruits (Figure 1). Adults also consume them, but mostly in periods of food shortage. The fruits are about 25mm in diameter. They have a thin, tender skin and a juicy melting flesh with an aroma and flavour faintly suggestive of apricots. When fully ripe they can be cooked to make a pleasant tasting jelly (Gonzalez Fischer and Garnett, 2016). The roots and stems are used for medicinal purposes, as a tonic, cooked and eaten as a soup.



Figure 1: Picture of Indigenous Fruits

2.5. Soil Fertility in Ethiopia

Soil fertility is a function of soil physicochemical and biological properties on which it can have qualitative and quantitative information to formulate the appropriate fertility management and agricultural practices (Mulugeta and Kibebew, 2016). Although it omits the importance of soil

physical and biological conditions for crop productivity, it is a useful simplification and it is a major concern of the world, including Ethiopia, which is the second most populous African country (Wang *et al.*, 2009). High quality soils not only produce better crops and fiber, but also help to maintain natural ecosystems and air and water quality. Soil fertility maintains and the nutrient balances are taken as key indicators of soil quality (Griffiths *et al.*, 2010).

But, decline in soil fertility implies reduction of quality of the soil, and soil fertility is defined as the decline in soil physical and chemical soil fertility, or a decrease in the levels of soil OM, N, pH, cation exchange capacity, exchangeable basic cations, P and adverse alteration of others physicochemical and biological properties of soil with the soil depth (Tufa *et al.*, 2018). Due to the continuous cultivation, long term usage of agrochemicals pesticides and chemical fertilizer, removal of crop residues and erosion problems the Ethiopian agricultural lands was losses in average 41 kg/ha of N, 6 kg/ha P and 26 kg/ha of K (Nega and Heluf, 2013). The adversely change of soil properties and soil fertility reduction are ultimately leads to affect the activities of soil microorganisms and availability of plant nutrients which in turns affect the crop productivity (Mengistu *et al.*, 2017).

In Ethiopia, the present decreasing of soil fertility is a major problem to carry out the proper agricultural practices on different land use types and to bring sustainable productivity in order to feed the ever increasing population of the country. In many cases, removals of vegetation cover, depletion of soil nutrients and OM, and accelerated soil erosion have all leads to change of physico-chemical properties of soil under different land use types and decreases in soil productivity (Ufot *et al.*, 2016).

2.6. Physical and Chemical Properties of Soil

The physical properties of soils determine the different function of soil and vary activities upon the soil resources. It determines their adaptability to cultivation and the level of biological activity that can be supported by the soil (Iqbal *et al.*, 2012). Soil texture is the proportion of sand, silt and clay particles with varying range of their size in diameter. Sand particles range in size from 0.05 to 2.00 mm, silt ranges from 0.002 to 0.050 mm, and the clay fraction is made up of particles smaller than 0.002 mm in diameter. Particles larger than 2.0 mm are referred to as

rock fragments and are not considered in determining soil texture, although they can influence both soil structure and soil water relationships (Brady and Weil, 2008). Soil texture is an important soil physical characteristic because it in part, determines water intake rate (infiltration), water storage in the soil, the ease of tilling and/or workability of the soil, the amount of aeration (vital to root growth), susceptibility to erosion, cation exchange capacity, pH buffering capacity and also influence soil fertility (Tufa *et al.*, 2018). Soil bulk density expresses the ratio of the mass of dry soil to the total volume occupied in the soil. The total volume includes both the solids (mineral and organic) and the pore spaces. Soil bulk density is an important soil physical property because it is an indicator of the soils porosity and soil compaction (Lechisa *et al.*, 2014).

The porosity of a soil is defined as the volume percentage of pores in a soil, i.e. the inverse of the volume percentage of solids. Within a soil of similar texture, a compacted soil has lower porosity and thus a greater bulk density, while a loose soil has a greater porosity and a lower bulk density (Teshome *et al.*, 2013). The total porosity of the soils usually lies between 30% and 70%, and may be used as a very general indication of the degree of compaction in a soil in the same way as bulk densities are used. The soils which have same particle, density the lower bulk density and it has higher total porosity (Nega and Heluf, 2013). It's the most important among the factors that determine the nutrient supplying power of the soil to the plants and microbes.

Soil chemical properties are the result of different soil minerals disintegration, decomposition and weathering of soil parent materials over the geological period (Ufot *et al.*, 2016). Soil pH or an indicator of soil acidity or alkalinity and is measured in pH units. The pH scale goes from 0 to 14 with pH 0 to 7 is acid, 7 to 14 basic and 7 as the neutral Point. A pH range of 6 to 7 is generally more favorable for plant growth because most plant nutrients are readily available in this range. However, some plants have soil pH requirements above or below this range (Fageria and Baligar, 2008). The pH value(s) of soil helps to identify the kinds of chemical reactions that are likely taking place in the soil and the possible agricultural practice carried on (Mengistu *et al.*, 2017). Electrical conductivity is a measure of salinity of the soil and thus to measure the total dissolved salts in the soil, the electrical conductivity measurement is sufficiently accurate for most purposes in agricultural practices (Alemayehu and Sheleme, 2013). Excessive accumulation

of soluble salts converts soils to salt affected soils and the process leading to accumulation of salts in arid and semi-arid regions where rainfall amount is insufficient to leach soluble salts (Qadir *et al.*, 2014).

Soil organic matter (OM) consists of dead plants, animal, microbes and fungi or their parts, as well as animal and microbial waste products in various stages of decomposition. Eventually, all of these break down into humus, which is relatively stable in the soil (Getahun and Bobe, 2015). Soil organic matter (OM) is considered to be a key attribute of soil quality and is usually considered as one of the most important properties of soils because of its impact on ecosystem sustainability and its effects on other soil physical, chemical, and biological characteristics (Abebe and Endalkachew, 2012). Organic matter improves soil structural stability in addition to providing mineral nutrients for plants and microorganisms through the mineralization process or biochemical oxidation of organic substrates (Tilahun and Asefa, 2009).

Chapter Three

3. Material and Methods

3.2. Description of the study area

The study was conducted in at specific place of Bura Adele and Deneba Kebele. Dodola woreda is located in Southeastern Ethiopia in the Oromia Regional State of the West Arsi Zone in the Dodola district. It is located between 735,000 UTM N-780000 UTM N latitudes and 480,000 UTM E-540000 UTM E longitudes (Figure 2). It is about 325 km from Addis Ababa (the capital city of Ethiopia) and 70 km from Shashemene town. The altitudinal range of the forest is from 2400 to 3712 m a.s.l. The daily temperature varies between 9 and 26 °C and the annual rainfall varies between 805 and 1260 mm (Mekonnen and Girma, 2023). The major economic activities of woreda are production of crop like wheat, barely, teff, vegetables and livestock's.

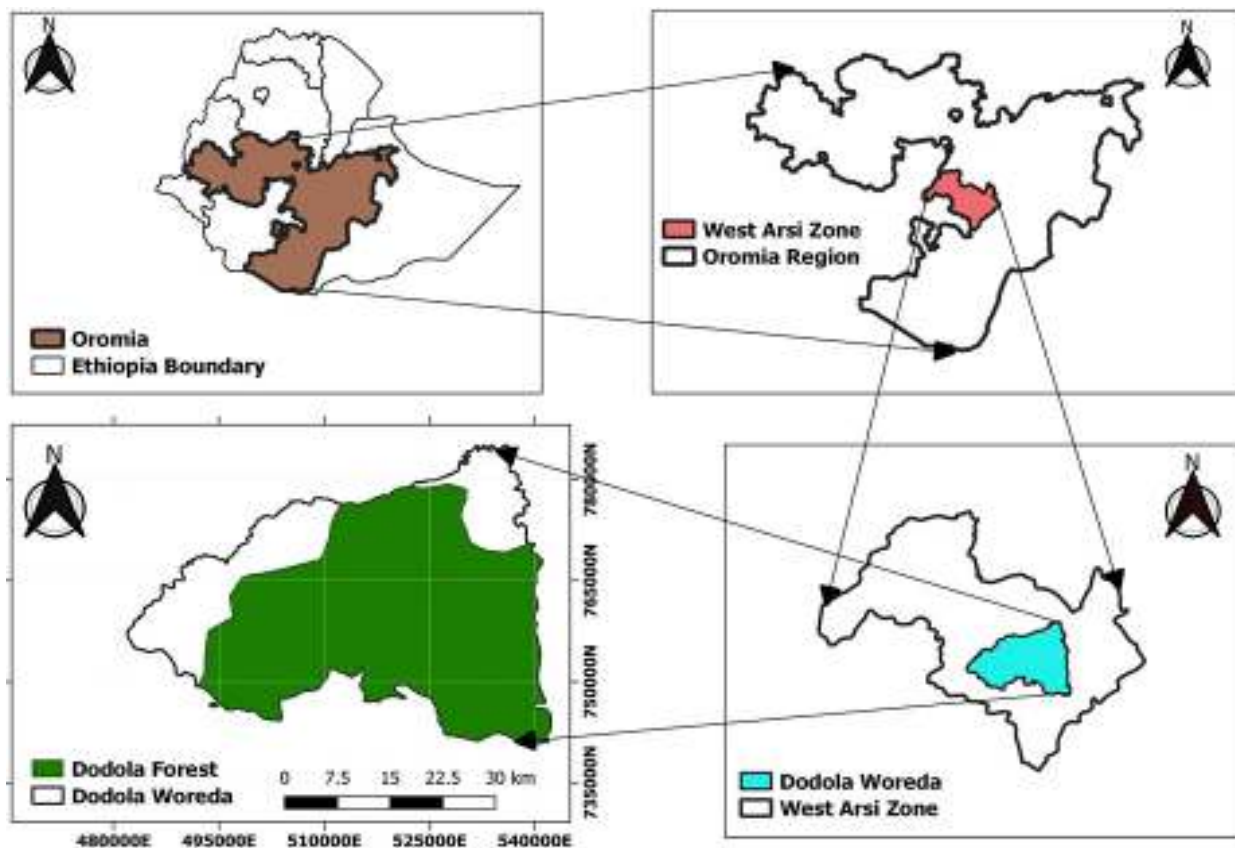


Figure 2: Map of the study area

3.3. Chemical and Instruments

3.3.1. Chemicals and reagents

All chemicals and reagent: analytical grade. 98% H₂SO₄ (Analytical reagent, India), 69.5% HNO₃ (Analytical Grade, German) and 30% H₂O₂ (Sigma Aldrich, England) was used for the digestion of *R. abyssinica* and *D. abyssinica* fruit. An intermediate standard solution of 10mg/L for each investigated metal was prepared from a 1000mg/L stock solution of the respective metal from which the calibration standards each metal was prepared and distilled water was used in the study.

3.2.2. Instrument and Apparatus

Atomic absorption spectrophotometer was used to determine concentration of each metal in *R. abyssinica* and *D. abyssinica* fruit. All samples were weighed by analytical balance with 120.00 g loading capacity and ± 0.0001 precision (ADAM, Model AFP-110L, England). Refrigerator (Oscar, Germany) was used to maintain temperature of sample. Hot plate/ digital water bath shaker was used for digesting sample on conical flask, measuring cylinders, pipettes were used for measuring different volumes of sample solution, acid reagents and organic acid standard solutions. Volumetric flasks were used for solution preparation, Electric grinder was used for grinding *R. abyssinica* and *D. abyssinica* fruit sample, aluminum foil, Whatman filter paper (No.42), knife, spatula, glass tube, wash bottle, hot plate, pestle and mortar was used for the study.

3.4. Sample Collection and Sample Preparation

2 kg of *R. abyssinica* and *D. abyssinica* fruit and soil samples were collected from each two selected kebeles (Bura Adele and Deneba) in dodola woreda. Dodola woreda was purposively selected depend on the availability of indigenous fruits. Then each sample was labeled and packed in polyethylene plastic bags and transported to laboratory for sampling. The composite samples was washed with tap water, then laid on a plastic flat sheet on the floor of a clean house and left for a week for air drying at room temperature. Then, the whole fruits of *R. abyssinica* and *D. abyssinica* fruit was peeled to separate from the stone. Whereas fruit samples was collected following removing the hard seed coat. All species-specific fruit samples was cut into slices and separately spread on plastic flat-bottom bowls for dry under room temperature (Natol *et al.*,

2023). Finally, dry the species-specific composite fruit samples was ground to a fine powder in a mixer grinder and sieve through the mesh and used for digestion analysis.

3.5.Digestion of Indigenous Fruit

0.5g each powder of *R. abyssinica* and *D. abyssinica* fruit and homogenized sample were transferred into each 100ml beaker and then 8ml of HNO₃ and 2ml of 30% H₂O₂ was added. Then each mixture was digested on hot plate at the temperature of 100°C for 3h. After sample digestion the digested solution was allowed to cool and 20ml distilled water was added, filtered with filter paper (42 mm) into 50ml volumetric flask, and then after that distilled water was added to filled until the mark. A reagent blank was also digested in parallel with the sample keeping all digestion parameters the same (Emine, 2018). Finally, each digested samples was directly aspirated to AAS for analysis the amount of each metal in *R. abyssinica* and *D. abyssinica* fruit sample.

3.6.Instrument operating conditions

Intermediate standard solutions containing 10mg/L was prepared from standard stock solutions that contain 1000mg/L. These intermediate standards was diluted with distilled water to obtain eight working standards for each metals (Zn, Fe, Cu, Mn, Co, Cd and Pd). Seven metals was determined using atomic absorption spectrophotometer (AAS) for simultaneous determination of all analyte wavelengths of interest. The AAS was determining all metals with replicates to measure the analytical signal.

3.7.Method of Validation

The linearity of the calibration curves was obtained by the value of coefficient (R^2) and the precision was expressed by the standard deviation of replicate measurements. The method limit of detection for each metal, and accuracy of the method was also described by the percent recovery for spike samples to validate the applicability of the method for determination of the studied metals in the fruits (Zelalem *et al.*, 2022).

3.7.1. Instrument calibrations

To calibrate the instrument using the calibration curve, four series of standard solutions were prepared for each metal through serial dilution of the respective stock solution (1000ppm). The linearity of the established calibration curves was evaluated by regression analysis. The intensity reading and concentrations of standards was used to calculate correlation coefficient (R^2) values for each metal.

3.7.2. Precision and Accuracy

Precision and accuracy of the analytical method was evaluated by repeatability and recovery studies of matrix spike (MS). A recovery study was performed by spiking three replicates of *Rhus abyssinica* and *Diospyros abyssinica* fruit samples with a known concentration of a metal standard solution (mid-range calibration concentration). The spiked samples were then subjected to the same digestion procedure like the actual sample. Precision was expressed as relative standard deviation (RSD) of the three replicate results and calculated as

Shown Eq.1:

$$RSD\% = \frac{S}{\bar{X}} \times 100 \quad \text{Eq. 1}$$

Where RSD is relative standard deviation, S is standard deviation and $\bar{X}$ is mean of measurements.

Accuracy is expressed as matrix spike recovery and the percent recovery data was calculated by the following equation 2 (Islary *et al.*, 2016)).

$$\% \text{Recovery} = \frac{C_{\text{Spike}} - C_{\text{Unspike}}}{C_{\text{added}}} \times 100 \quad \text{Eq. 2}$$

3.7.3. Limit of Detection (LOD)

Method detection limit is the smallest concentration of analyte that can be distinguished from statistical fluctuations in the blank, which usually corresponds to the standard deviation of the blank solution times a constant. In this study, the digested blank solutions containing HNO_3 and H_2O_2 then analyzed by AAS triplicate readings was obtained for each blank and the standard deviation of the blank solution was calculated. The method of detection limit of each element

was obtained by multiplying the three times standard deviation of the reagent blank (Eq.3)(Haile *et al.*, 2018).

$$LOD = 3S_b \quad \text{Eq. 3}$$

Where: S_b is standard deviation of blank reagent readings

3.7.4. Limit of Quantification (LOQ)

Limit of quantification is the lowest concentration at which a measurement is quantitatively meaningful. The LOQ is most often defined as 10 times the signal/noise ratio plus the mean concentration of blank if the noise is approximated as the standard deviation of the blank. In this study, LOQ was obtained from triplicate analysis of reagent blanks which was digested in the same digestion procedure for fruit and soil samples. The LOQ was calculated by multiplying 10 times of standard deviation of the reagent blank shown Eq.4.(Hagos and Chandravanshi, 2016).

$$LOQ = 10S_b \quad \text{Eq. 4}$$

3.8. Statistical analysis

The preparation of calibration curves and data analysis was carried out using Microsoft Excel 2013. To validate and compare the average values of the metals across various sampling sites, a one-way ANOVA was employed.

Chapter Four

4. Results and Discussion

4.2.Validation Method

4.2.1. Calibration method

Table 1 shows that correlation coefficients(R^2) of the calibration curve for the entire metals were greater than or equal to 0.99 which assured the linearity of instrumental response for individual analytes.

Table 1: Working standard concentration and correlation coefficients

Metals	Working standard concentration (ppm)	Wavelength	R^2
Fe	0.05, 1.0, 2.0, 4.0	248.3 nm	0.9984
Zn	0.01, 0.1, 1, 2	213.9	0.9945
Mn	0.03, 0.1, 0.4, 0.8	279.5	0.9905
Cu	0.006, 0.1, 0.3, 0.5	324.7	0.9935
Co	0.1, 1, 2, 3	240.7	0.9963
Cd	0.015, 0.04, 0.06, 0.1	283.2	0.9937
Pb	0.05, 0.1, 0.2, 0.3	228.9	0.9986

4.2.2. Method of Detection and Quantification limit

Table 2 showed that limit of detection of ranged from 0.003-0.006 for *Rosa abyssinica* and 0.001-0.018 mg/kg *Dovyalis abyssinica* while the limit of quantification ranged from 0.03-0.06 mg/kg for *Rosa abyssinica* and 0.01-0.18 mg/kg for *Dovyalis abyssinica*. The finding of the study revealed that limit of detection and quantification greater than instrumental detection limit (0.0001-0.0004 mg/kg) for both plants was indicated that the proposed analytical method was reliable and accurate for the analysis of heavy metals in the selected plants. The finding of the study is in agreement with the literature reported by (Soetan et al., 2010).

Table 2: Method of detection limit and quantification limit of each plants for analyzed metals by AAS (mg/kg)

Metals	MDL of <i>Rosa abyssinica</i>	MDL of <i>Dovyalis abyssinica</i>	MLQ of <i>Rosa abyssinica</i>	MLQ of <i>Dovyalis abyssinica</i>	IDL
Fe	0.003	0.009	0.03	0.09	0.0001
Zn	0.003	0.003	0.03	0.03	0.0003
Mn	0.003	0.018	0.03	0.18	0.0004
Cu	0.003	0.018	0.03	0.18	0.0003
Co	0.003	0.003	0.03	0.03	0.0002
Cd	0.006	0.003	0.06	0.03	0.0001
Pb	0.002	0.001	0.06	0.01	0.0003

4.2.3. Accuracy and Precision

Table 3 and Table 4 showed that efficiency and reproducibility of the selected method. The recovery values for the range of 88.7% to 119.3% and 90-120% which are in acceptable range of 80-120 %.The reproducibility of the analytical procedure was checked by carrying out a triplicate analysis and calculating the relative standard deviation (%RSD) for each metal. In the precision test, %RSD for selected essential and non-essential metals *Rosa abyssinica* and *Dovyalis abyssinica* was below 10% the mean which indicated that the analytical method used is precise and reliable.

Table 3: Recovery test of *Rosa abyssinica* for each metals (mg/kg)

Metals	Unspiked	Amount added	Spiked	Recovery (%)	RSD%
Fe	232.8±0.8	60	286±1	88.7	0.40
Zn	84.5±1	10	95.5±0.2	110.0	0.20
Mn	4.87±0.02	10	14.3±0.15	94.3	1.00
Cu	56.3±0.1	10	56.4±0.15	100.0	0.30
Co	0.1±0.006	1	1.14±0.01	104.0	0.90
Cd	0.027±0.006	1	1.22±0.01	119.3	0.80
Pb	0.07±0.006	1	1.04±0.01	97.0	0.96

Table 4: Recovery test of *Dovyalis abyssinica* of each metals (mg/kg)

Metals	Unspiked amount	Amount added	Spiked amount	Recovery (%)	RSD%
Fe	273±1	10	282±0.001	90.0	0.00035
Zn	86.3±0.6	10	97.4±0.400	111.0	0.41
Mn	4.92±0.01	10	15.1±0.100	101.8	0.70
Cu	63.7±0.1	1	64.7±0.100	,100.0	0.20
Co	0.1±0.00	1	1.3±0.010	120.0	0.80
Cd	ND	-	-	-	
Pb	ND	-	-	-	

4.3.Distribution of Essential and Non-essential metals

The levels of essential and non-essential metals in the *Rosa abyssinica* and *Dovyalis abyssinica* fruit were presented in Table 5. The level of Fe, Zn, Mn, Cu and Co was higher in *Dovyalis abyssinica* fruit than *Rosa abyssinica* fruit. (Ayenew *et al.*, 2014; Minaleshewa *et al.*, 2010; Sailus, 2014). The other probable reason for the higher concentrations of Fe, Zn, Mn, Cu and Co in *Dovyalis abyssinica* fruit is due to the presence of enrichment of organic matter in the soil which is rich with these metals (Weeraratna *et al.*, 1977). The mean concentration of Fe in *Rosa abyssinica* and *Dovyalis abyssinica* fruits were ranged from 232.8 ±0.01 mg/kg and 273±1 mg/kg respectively. The highest mean concentration was found in *Dovyalis abyssinica* and the

lowest concentration was found in *Rosa abyssinica*. The concentration of Fe found in this study was slightly higher than the value of 2.88 mg/kg reported. This might be due to geographical and geological differences of the soil. The permissible limit of Fe set by FAO/WHO in edible plants was 425.5 mg/kg. ANOVA analysis showed that the mean concentrations of Fe in *Rosa abyssinica* and *Dovyalis abyssinica* were significantly different from each other. As indicated in Table 5, the level of Zn in *Rosa abyssinica* and *Dovyalis abyssinica* was ranged from 5.30 to 10.09 mg/kg. The highest mean concentration of Zn was found 84.5 ± 1 mg/kg and 86.3 ± 0.6 mg/kg respectively. The concentration of Zn was highest in *Dovyalis abyssinica* while the lowest concentration was observed in *Rosa abyssinica*. The levels of Zn in plants 13.93 mg/kg have been reported higher than obtained in this study. One-way analysis of variance showed that the mean concentration of Zn in *Rosa abyssinica* and *Dovyalis abyssinica* were significantly different ($p < 0.04$). The level of Zn observed in both plant was below the FAO/WHO permissible limit. The concentration of Mn in *Rosa abyssinica* and *Dovyalis abyssinica* was found out 4.87 ± 0.02 mg/kg and 4.92 ± 0.01 mg/kg respectively. The highest level of Mn was observed in *Dovyalis abyssinica* and the lowest concentration was found in *Rosa abyssinica*. The value (184 - 401 mg/kg) reported by Yohannes and Chandravanshi (2015) was higher than the value obtained in this study. The concentration of Cu was found 56.4 ± 0.1 and 63.6 ± 0.1 mg/kg respectively. The concentration of Cu in *Rosa abyssinica* and *Dovyalis abyssinica* were found to mg/kg, which falls below the safe limits for human health. The mean concentration of Co in *Rosa abyssinica* and *Dovyalis abyssinica* fruit was 0.1 ± 0.006 and 0.1 ± 0.001 mg/kg respectively.

Nonessential metals (Cd and Pb) were found below the FAO/WHO limits in *Rosa abyssinica* while the concentration Cd and Pb were not detected in *Dovyalis abyssinica*. The absence of Pb and Cd may be attributed to the fact that no commercial fertilizers, pesticides, or herbicides are used for *Rosa abyssinica* and *Dovyalis abyssinica* plantations in the study area, ensuring a more natural and pristine growing environment. ANOVA analysis revealed that the concentrations of Fe, Zn, Mn, and Cu were significantly different between the two plants fruits, while there was no significant difference in the concentrations of Co, Cd, and Pb between the plants fruits.

Table 5: Concentration of essential and non-essential metals

Metals	<i>Rosa abyssinica</i> Fruit	<i>Dovyalis abyssinica</i> Fruit	FAO/WHO limits
Fe	232.8±0.8	273±1	425.5
Zn	84.5±1	86.3±0.6	99.4
Mn	4.87±0.02	4.92±0.01	5
Cu	56.3±0.1	63.6±0.1	73.3
Co	0.1±0.006	0.1±0.001	0.15
Cd	0.027±0.006	ND	0.2
Pb	0.07±0.006	ND	0.3

Chapter Five

5 Conclusion and Recommendations

5.1. Conclusion

Determination of essential and nonessential metals concentration in indigenous plant is important for health risk assessment during food consumption. The metals such as Fe, Zn, Mn, Cu, Co, Cd and Pb were analyzed by using atomic absorption spectrometry in the *Rosa abyssinica* and *Dovyalis abyssinica* fruit in the Dodola Woreda, Ethiopia. The validation method of digestion process produced good recovery results ranged from 88.7% up to 119.3% for *Rosa abyssinica*, while 90 up to 120 % for *Dovyalis abyssinica* with %RSD below 10% which shows the efficiency of method used. The experimental results of each fruit, the mean concentration of essential and non-essential metals (mg/kg) in *Rosa abyssinica* sample exhibited decreased trend in the order of Fe (232.8 ± 0.8) > Zn (84.5 ± 1) > Mn (4.87 ± 0.02), Cu (56.4 ± 0.1), Co (0.1 ± 0.006), Cd, (0.027 ± 0.006) > Pb (0.07 ± 0.006), while mean concentration for the *Dovyalis abyssinica* samples follow the decreasing order of Fe (273 ± 1) > Zn (86.3 ± 0.6) > Mn (4.92 ± 0.01), Cu (63.6 ± 0.1), Co (0.1 ± 0.001), Cd, (ND), Pb (ND). Slightly a significant difference was observed between the mean concentrations of all metals in *Rosa abyssinica* and *Dovyalis abyssinica* fruit. This indicates that the samples contain higher levels of selected essential metals compared to non-essential metals in both plants. In the precision test, the %RSD (relative standard deviation) for the selected essential and non-essential metals was below the recommended level. Therefore, the content of essential and non-essential metals in both indigenous plant fruits was below the permissible value of WHO/FAO and can be safely edible.

4.4.Recommendations

Based the finding and the conclusion drawn above, the following recommendations are forwarded.

- ✓ Monitoring of the levels of some essential and non-essential metals in differentindigenous plant should be encouraged.
- ✓ We recommend that other metals, in addition to those studied, should be investigated in these indigeneous plants.

5. References

- Afshin, A.; Sur, P. J.; Fay, K. A.; Cornaby, L.; Ferrara, G.; Salama, J. S.; et al. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 2019, 393(10184), 1958-1972.
- Ayenew, A.; Gebremariam, B.; Ephrem, E. Levels of Essential and Toxic Metals in Ethiopian Khat, (*Catha edulis* Forsk.). *Ethiopian Journal of Environmental Studies & Management* 2014, 7, 289-297. <https://doi.org/10.4314/ejesm.v7i3.8>
- Minaleshewa, A.; Chandravanshi, B.S.; Mesfin, R. Concentration Levels of Essential and Non-Essential Metals in Ethiopian Khat (*Catha edulis* Forsk). *Biological Trace Element Research* 2010, 138, 316-325. <https://doi.org/10.1007/s12011-010-8617-1>
- Alemayehu, K.; Sheleme, B. Effects of different land use systems on soil physical and chemical properties in Sodo Zuria Woreda of Wolaita zone Southern Ethiopia. *Journal of Soil Science and Environment Management* 2013, 4(5), 100-107.
- Getahun, B.; Bobe, B. Impacts of land use types on selected soil physicochemical properties of Loma Woreda, Dawuro Zone, Southern Ethiopia. *Science, Technology and Arts Research Journal* 2015, 4(4), 40-48.
- Lechisa, T.; Chimdi, A.; Abebaw, A. Dynamics of soil fertility as influenced by different land use systems and soil depth in west Showa Zone, Gindeberet district, Ethiopia. *Agriculture Forestry and Fisheries* 2014, 3(6), 489-494.
- Griffiths, B.; Ball, B.; Bohanec, M. Integrating soil quality changes to arable agricultural systems following organic matter addition, or adoption of a ley-arable rotation. *Applied Soil Ecol.* 2010, 46(1), 43-53.
- Brady, N.; Weil, R. *The Nature and Properties of Soil, 14th edition*. Upper Saddle River, NJ: Prentice Hall, 2008.
- Mengistu, C.; Kibebew, K.; Tarekegn, F. Influence of different land use types and soil depths on selected soil properties related to soil fertility in Warandhab area, Horo Guduru Wallaga Zone, Oromiya, Ethiopia. *International Journal of Environmental sciences and natural Resources* 2017, 4(2), 1-11.
- Mulugeta, A.; Kibebew, K. Assessment of soil fertility status at Dawja Watershed in Enebe Sar Midir district, Northwestern Ethiopia. *International Journal of Plant and Soil Science* 2016, 11(2), 1-13.

- Nega, E.; Heluf, G. Effect of land use changes and soil depth on soil organic matter, total nitrogen and available phosphorus contents of soils in senbat watershed, western Ethiopia. *Asian Research Publishing Network Journal of Agricultural and Biological Science* 2013, 8(3), 206-212.
- Qadir, M.; Quillerou, E.; Nangia, V.; Murtaza, G. C. P.; Noble, A.D. Economics of salt-induced land degradation and restoration. National Resources Forum, United Nations, 2014. DOI: 10.1111/1477-8947.12054.
- Teshome, Y.; Heluf, G.; Kibebew, K.; Sheleme, B. Impacts of land use on selected physicochemical properties of soils of Abobo Area, Western Ethiopia. *Agriculture, Forestry and Fisheries* 2013, 2(5), 177-183.
- Tufa, M.; Asmare Melese, A.; Tena, W. Effects of land use types on selected soil physicochemical properties: the case of Kuyu district, central highland of Ethiopia. Master thesis, Debre Berhan University, Ethiopia, 2018.
- Ufot, U.; Iren, O.; Chikere Njoku, C. Effects of land use on soil physical and chemical properties in Akokwa area of Imo State, Nigeria 2016, 2(3), 273-278.
- Sailus M.I. Determination of the Levels of Selected Heavy Metals in Soil and Khat (*Catha edulis* Forsk) Grown in Kenya. MSc. Thesis, Kenyatta University, Nairobi, 2014.
- Weeraratna, C.S., Watson, M. and Wettasingha, D.T. Effect of Mineralization of Tea Pruning on Some Soil Characteristics. *Plant and Soil* 1977, 46, 93-99.
- Demisew, J.; Chandravanshi, B. Mineral contents of Ethiopian red and green apple fruits: A comparison with WHO/FAO standards. *Chem. Int.* 2021, 7, 112-122.
- Dessie, A. T.; Minbale, A. T. Determination of the Levels of Selected Essential Metals in Sycamore (*Ficus sycomorus* L) Fruit and Seed Using Flame Atomic Absorption Spectrophotometry. *Journal of Chemistry* 2023, 11.
- Emine, N. Biochemical characterization of fig (*Ficus carica* L.) seeds. *Journal of Agricultural Sciences* 2018, 25, 232-237.
- Soetan K.; Olaiya C.; Oyewole O. The importance of mineral elements for humans, domestic animals and plants. *African Journal of Food Science* 2010, 4(5), 200-222.

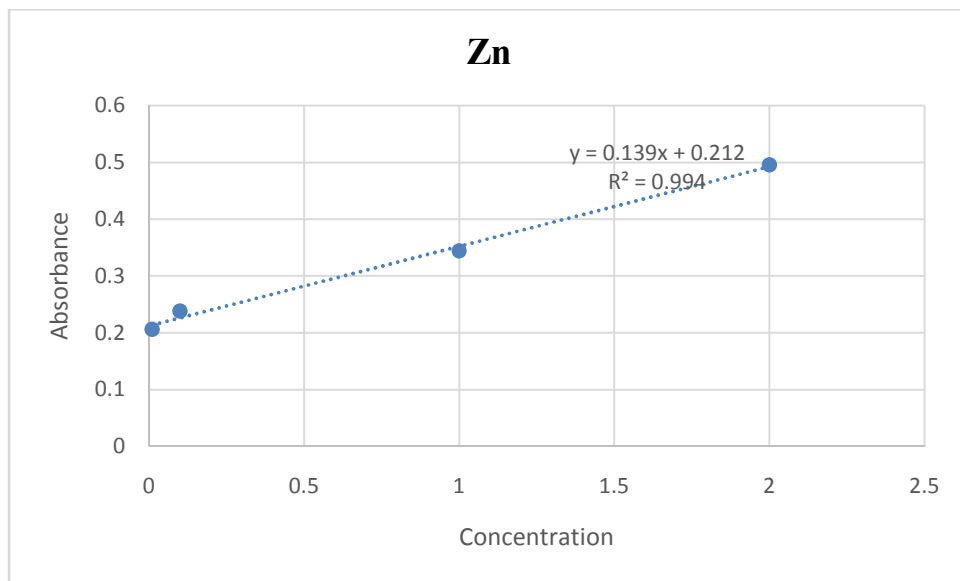
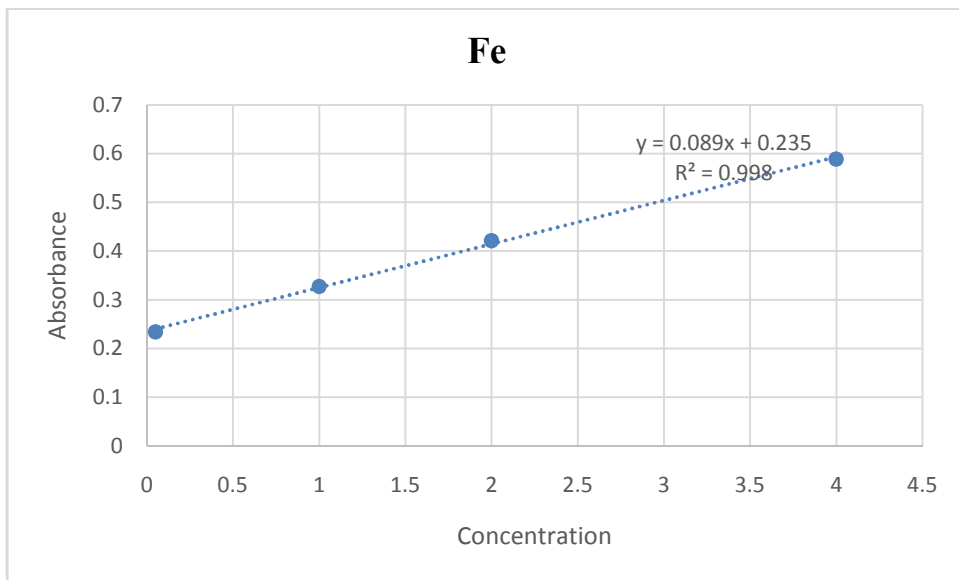
- Gebrelibanos, M.; Megersa, N.; Taddesse, A. M. Levels of essential and non-essential metals in edible mushrooms cultivated in Haramaya, Ethiopia. *International Journal of Food Contamination* 2016, 3(2), 1-12.
- Wang, Z.; Song, K.; Zhang, B.; Liu, D.; Li, X.; Ren, C. Spatial variability and affecting factors of soil nutrients in croplands of Northeast China: a case study in Dehui county. *Plant Soil Environ.* 2009, 55, 110-120.
- Iqbal, M.; Khan, A.; Raza, K.; Amjad, M. Soil physical health indices, soil organic carbon, nitrate contents and maize growth as influenced by dairy manure and nitrogen rates. *International Journal of agriculture and Biology* 2012, 14, 20-28.
- Gonzalez Fischer, C.; Garnett, T. Plates, pyramids, planet: Developments in national healthy and sustainable dietary guidelines: a state of play assessment. Food and Agriculture Organization of the United Nations and The Food Climate Research Network at The University of Oxford 2016.
- Graham, D.; Welch, R.M.; Saunders, D.A.; Ortiz, M.B.; Bonierbale, M.; Dehaan, S.; Burgos, G.; Thiele, G.; Liria, R.; Beebe, S.E. Nutritious subsistence food systems. *Advances in Agronomy* 2007, 92, 1-74.
- Hagos, M., Chandravanshi, B.S. Levels of essential and non-essential metals in fenugreek seed (*Trigonella Foenum-Graecum* L.) cultivated in different parts of Ethiopia. *Brazilian Journal of Food Technology* 2016, 19, e2015059.
- Haile, E.; Tesfau, H.; Washe, A. P. Determination of dietary toxins in selected wild edible plants of Ethiopia. *Food Science and Quality Management* 2018, 75, 1-9.
- Islary, A.; Sarmah, J.; Basumatary, S. Proximate composition, mineral content, phytochemical analysis and in vitro antioxidant activities of a wild edible fruit (*Grewia sapida* Roxb. ex DC.) found in Assam of North-East India. *Journal of Investigative Biochemistry* 2016, 5(1), 21-31.
- Jemaneh, D.; Chandravanshi, B.S. Mineral contents of Ethiopian red and green apple fruits: A comparison with who/FAO standards. *Chemistry International* 2021, 7, 112-122.
- Kennedy, G.; Pedro, M.R.; Senhier, C.; Nantel, G.; Brouwer, I. (Dietary diversity score is a useful indicator of micronutrient intake in non-breast feeding Filipino children. *Journal of nutrition* 2007, 137, 1-6.

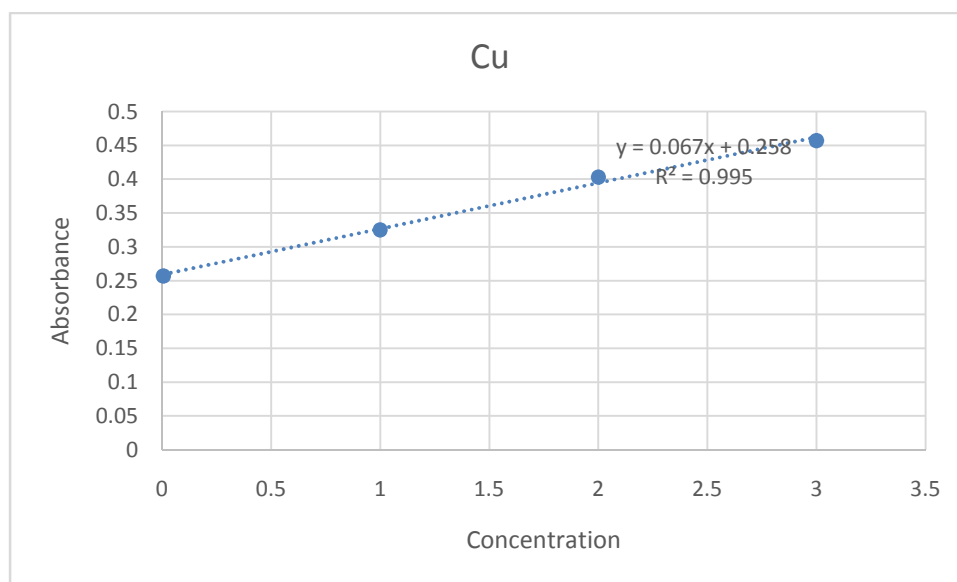
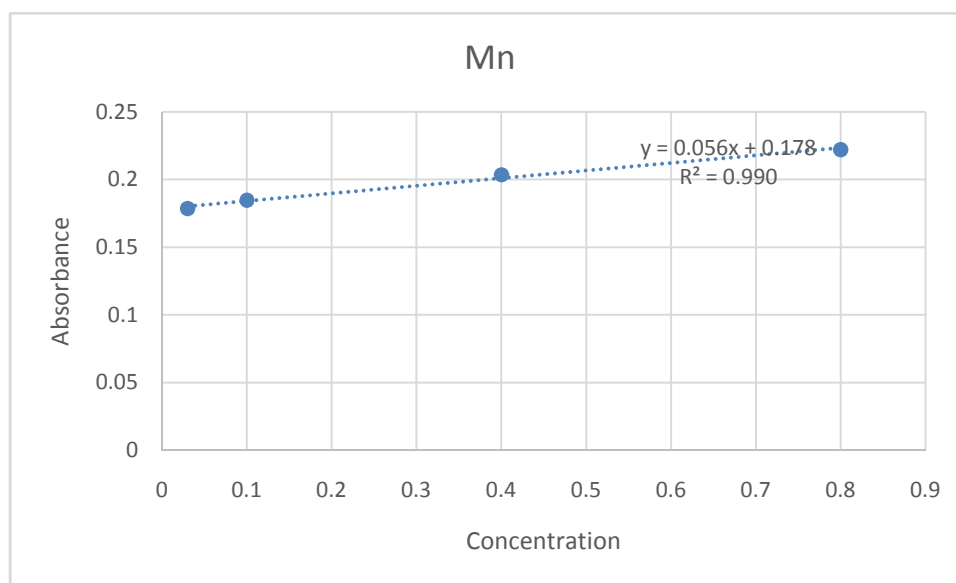
- Mebrahtu, H.; Chandravanshi, B. S. Levels of essential and non-essential metals in fenugreek seed (*Trigonella Foenum-Graecum* L.) cultivated in different parts of Ethiopia. *Braz. J. Food Technol.* 2016, *19*, e2015059.
- Mekonnen, G.; Girma, Z. The role of Dodola Community Conservation Area for large mammal conservation, Ethiopia. *BMC Research Notes* 2023, *16*, 378.
- Melaku, A.; Mohammed, A. E. Critical Review on Wild-Edible Fruit Species in Ethiopia. *International Journal of Forestry Research* 2021, *12*.
- Mulugeta, M.; Gebretsadik, Y.; Birhane, E.; McMullin, S.; Erick, N.; Kiros, M. H.; et al. Nutritional and Eco climatic importance of indigenous and naturalized wild edible plant species in Ethiopia. *Food Chemistry: Molecular Sciences* 2022, *4*, 100084.
- Natol, A. J.; Gemed, H. F.; Ebisa, O. K. Nutritional Composition and Antioxidant Properties of Selected Underutilized Wild Edible Fruits in East Wollega Zone, Western Ethiopia. *International Journal of Fruit Science* 2023, *23*(1), 34-45.
- Omari, R.; Quorantse, K. E.; Omari, P. K. Nutrition knowledge and food consumption practices and barriers in rural Ghana: The case of foods for preventing vitamin a and iron deficiencies. *Afr. J. Food Agr. Nutr. Dev.* 2017, *17*(1), 11639-11656.
- Sardeshpande, M.; Shackleton, C. Wild edible fruits: a systematic review of an under-researched multifunctional NTFP (non-timber forest product). *Forests*, 2019, *10*(6), 467.
- Shambel, G.; Chandravanshi, B.; Taddese, W.; Cherinet, A. Assessment of selected nutrients and toxic metals in fruits, soils and irrigation waters of Awara Melka and Nura Era farms, Ethiopia. *SpringerPlus* 2016, *5*, 747.
- Sibiya, N. P.; Kayitesib, E.; Moteetee, A. Mineral composition of selected indigenous wild southern African fruits. *South African Journal of Botany* 2020, *132*, 8794.
- Teklehaymanot, T.; Giday, M. Ethnobotanical study of wild edible plants of Kara and Kwego semi-pastoralist people in lower Omo river valley, Debub Omo zone, SNNPR, Ethiopia. *Journal of Ethnobiology and Ethnomedicine* 2010, *6*(1), 23-28.
- WHO. Trace elements in human nutrition and health, Geneva: 1996, 72-103.
- Zelalem, P.; Bhagwan, S. C.; Yohannes, W.; Embiale, A. Levels of selected metals in *Ficus sur* Forssk fruit and soil of the plant grown in different parts of Ethiopia. *Ethiop. J. Sci.* 2021a, *44*(1), 1-12.

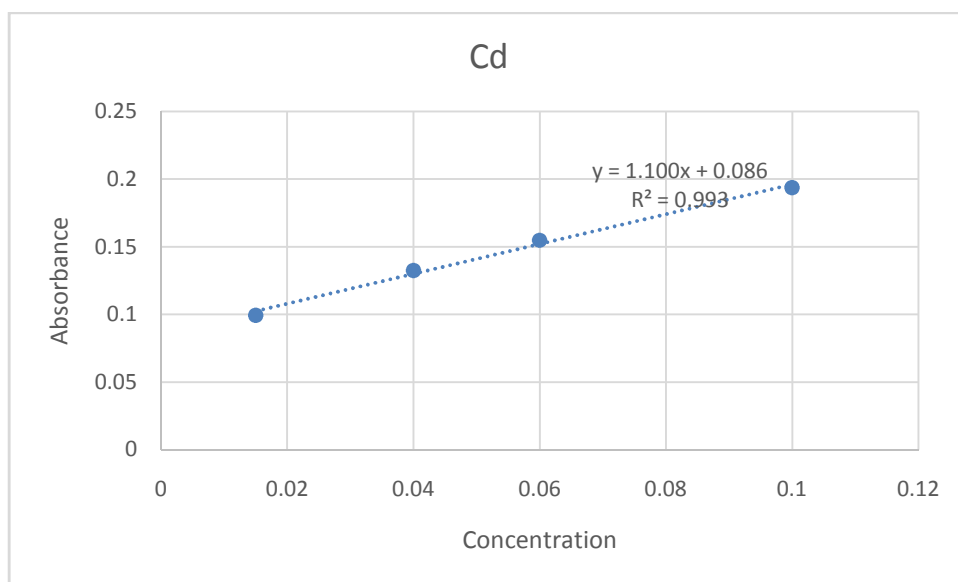
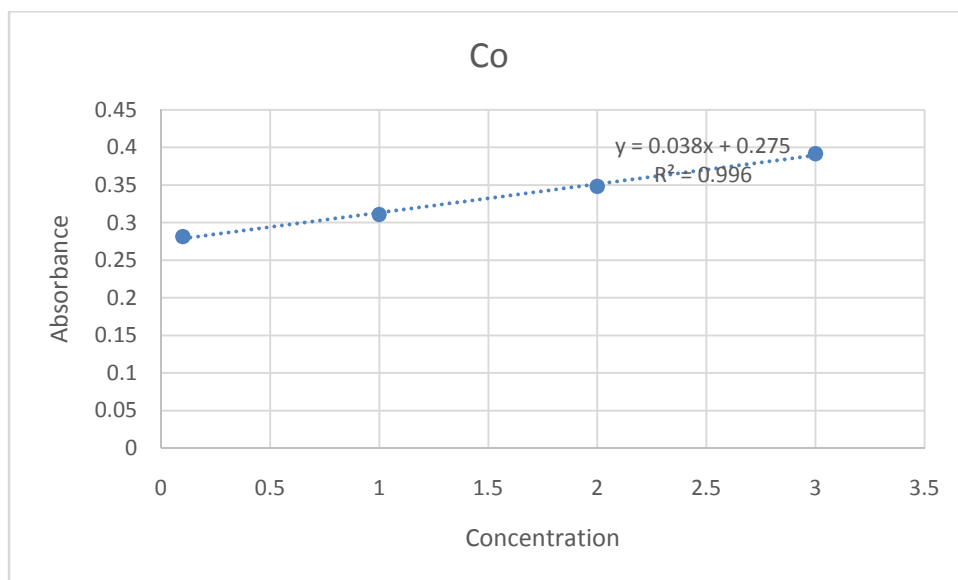
- Zelalem, P.; Bhagwan, S. C.; Weldegebriel, Y.; Asamene, E. Levels of selected metals in wild edible plant *Embelia schimperi* Vatke fruit under different agro-ecological zones, Ethiopia. *Chemistry International* 2022, 8(1), 1-11.
- Zelalem, P.; Chandravanshi, B. S.; Yohannes, W.; Embiale, A. Levels of selected metals in the fruits of a wild edible plant (*Rubus Steudneri* Schweinf) and its underlying Soil. *Bull. Chem. Soc. Ethiop.* 2021, 35(2), 217-228.
- Zemenay, Z. A.; Fikirte, Z. S.; Molaligne, M. T. Investigation of heavy metals in selected local spices consumed in Ethiopia. *Cogent Food and Agriculture* 2021, 7(1), 1917091.

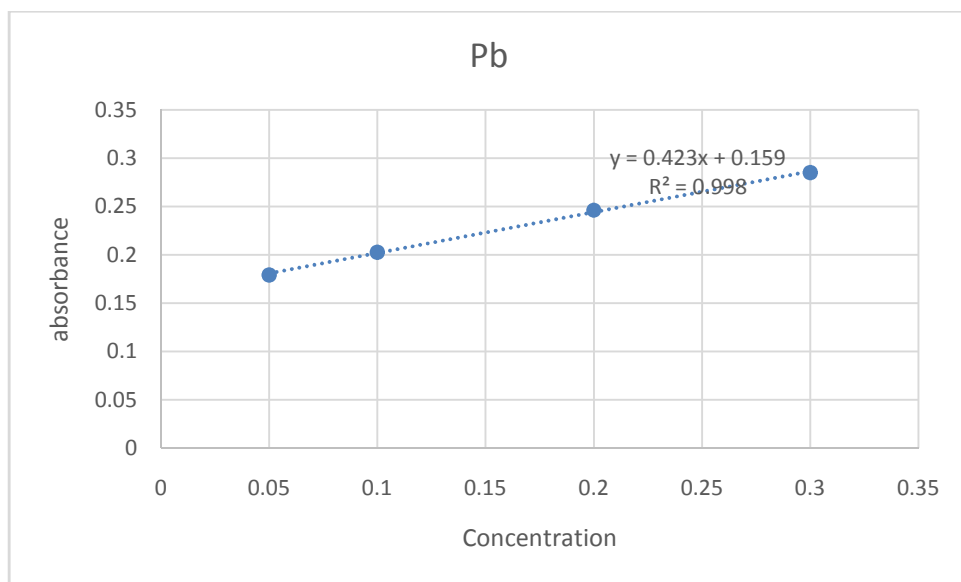
6. Appendix

Appendix I: calibration curve









Appendix II: Stastical analysis

Descriptive Statistics of blank

	N	Mean	Std. Deviation
FeRA	3	5.416667	.00115470
FeDA	3	6.836667	.00305505
ZnRA	3	.092000	.0010000
ZnDA	3	.243333	.00115470
MnRA	3	.086667	.0015275
MnDA	3	.103000	.0060828
CuRA	3	.078000	.0010000
CuDA	3	.843333	.0057735
CoRA	3	.089000	.0010000
CoDA	3	.950000	.00100000
CdRA	3	.067000	.0020000
CDDA	3	.056000	.0010000
PbRA	3	.076000	.0020000
PbDA	3	.084000	.0010000
Valid (listwise)	N3		

Descriptive Statistics of unspiked and spiked

	N	Mean	Std. Deviation
FeUs	3	232.8333	.76376
FeS	3	286.0000	1.00000
ZnUs	3	84.4633	.20502
ZnS	3	95.5000	.20000
Mnus	3	4.8700	.02000
Mns	3	14.3333	.15275
CuUs	3	56.4000	.10000

CuS	3	145.6667	.15275
CoUs	3	.1133	.00577
CoS	3	1.1400	.01000
CdUs	3	.0267	.00577
CdS	3	1.2200	.01000
PbUs	3	.0733	.00577
PbS	3	1.0400	.01000
Valid N (listwise)	3		

ANOVA of *Rosa abyssinica*

		Sum of Squares	df	Mean Square	F	Sig.
Fe	Between Groups	1.167	2	.583	0.40	0.000
	Within Groups	.000	0	.		
	Total	1.167	2			
Zn	Between Groups	.084	2	.042	0.32	0.040
	Within Groups	.000	0	.		
	Total	.084	2			
Mn	Between Groups	.001	2	.000	0.18	0.002
	Within Groups	.000	0	.		
	Total	.001	2			
Cu	Between Groups	.020	2	.010	0.15	0.001
	Within Groups	.000	0	.		

	Total	.020	2			
Co	Between Groups	.000	2	.000	0.000	0.07
	Within Groups	.000	0	.		
	Total	.000	2			
Cd	Between Groups	.000	2	.000	.000	0.08
	Within Groups	.000	0	.		
	Total	.000	2			
Pb	Between Groups	.000	2	.000	.000	0.06
	Within Groups	.000	0	.		
	Total	.000	2			

Appendix III: Experimental process





