HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTEMENT OF CHEMISTRY

MASTER'S THESIS ON

DETERMINATION OF SELECTED ESSENTIAL AND NON- ESSENTIAL METALS IN TEFF GRAIN SAMPLES COLLECTED FROM YILMANA DENSA WOREDA, WEST GOJAM ZONE, ETHIOPIA

BY: ZELALEM TALACHEW

ADVISOR: ADELEW ESTIFANOS (PhD)

NOVEMBER, 2024

HAWASSA, ETHIOPIA

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A THESIS SUBMITTED TO HAWASSA UNIVERSITY COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES DEPARTEMENT OF CHEMISTRY POST GRADUATE PROGRAM IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY, UNDER THE SUPERVISION OF Dr. ADELEW ESTIFANOS.

NOVEMBER ,2024

HAWASSA, ETHIOPIA

DECLARATION

I do here by declare that the thesis work entitled **"Determination of selected essential and non-essential metals in teff grain samples in Yilmana Densa Woreda, West Gojam Zone ''**, is my original work and has not been submitted for a degree or diploma in any other universities by any other researchers or any students and that all sources of materials used for this thesis have been duly acknowledged.

Name: Zelalem Talachew

Signature: ___________________________________

This thesis has been submitted for examination with my approval as thesis Advisor

SCHOOL OF GRADUATE STUDIES

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ADVISOR APPROVAL SHEET-1

This is to certify that the thesis entitled **“Determination of selected essential and non- essential metals in teff grain samples in Yilmana Densa Woreda, West Gojjam Zone ,Ethiopia”** submitted in partial fulfillment of the requirements for the degree Master of Science in Chemistry the graduate program of the Department of Chemistry, Hawassa University, and is a record of original research carried out by Zelalem Talachew (ID:- PGchemK/018/11) under our supervision, and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it is accepted as fulfilling the thesis requirements.

Adelew Estifanos (PhD)	___________________	_______________
Name of advisor	Signature	Date

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EXAMINER APPROVAL SHEET-2

We, the undersigned, members of the board of Examiners of the final defense by Zelalem Talachew have read and evaluated this thesis entitled **"Determination of selected essential and non- essential metals in teff grain samples in Yilmana Densa Woreda, West Gojjam Zone, Ethiopia"** examined the candidate. This is therefore to certify that, the thesis has been accepted for partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	**Analysis of variance**
AI	Adequate Intake
CSA	Central Statistical Authority
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agricultural Organization
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IOM	Institute of Medicine
IQ	Intelligence Quotient
RDA	Reference Dietary Allowance
% R	Percentage Recovery
R^2	Determination coefficient
USDA	United States Department of Agriculture
WHO	World Health Organization
YDWCSA	Yilmana denssa Woreda Centeral Statistical Agency

ABSTRACT

Teff [Eragrostis teff (Zuccagni) Trotter] is one of the major and indigenous cereal crops in Ethiopia. Teff is a staple food in Ethiopia, consisting of two-thirds of their cereal diet and is mainly used to make human food. Although Teff is farmed for its grain, the straw is also used to build homes in both rural and urban areas, to reinforce mud or plaster, and as feed for cattl.

The levels of selected essential and non-essential metals in the white, red, and mixed teff grains collected from Yilmana Densa Woreda, West Gojam Zone (Ethiopia) were determined by AAS equipped with deuterium background corrector and air-acetylene flame atomizer. After a suitable sample pretreatment, the powdered teff was wet digested with the acid mixture (8 mL HNO_3:2 mL H_2O_2) at 180 ^oC for 3:00 h over wet digestion block. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with a standard solution of metals, and the percentage recoveries varied from 99 % to 113%. The mean concentrations of metals determined (mg/kg, dry weight) were in the ranges of Fe (632-761) > Mn (191-516) > Zn (87-168) > Cu (75-112) > Ni (4.15-10.81) > Co (5.36-7.15) > Cr (3.93-5.15) > Pb (1.14-2.26). A statistical analysis of variance indicated that there is significant difference between the mean concentrations of all metals (Co, Cr, Pb Ni, Fe, Mn, Cu and Zn) with ($P < 0.05$ at 95% confidence interval) between the different types of teff samples. . The concentrations of the metals showed that the red teff samples collected from fetlo kuskaum have higher amounts of Fe and Cu, compared to that of the white and mixed teff samples. The mixed teff samples collected from Kililt has higher amounts of Fe, Mn and Zn, compared to that of the red and white teff samples.The amount of essential metals is high in red teff samples at Fetillo Kuskuam and in mixed teff at Kililt. The presence of significant differences may be due to the presence of different geographical distribution, rainfall, soil composition, harvesting, and storing methods.

KEY WORDS*:* Teff grain, Essential metals, Non-essential metals, Ethiopia, sample digestion

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

Teff [Eragrostis teff (Zuccagni) Trotter] is one of the major and indigenous cereal crops in Ethiopia, where it is believed to have originated[1]. It is considered a low risk crop from the perspectives that it can be cultivated in a broad range of ecological surroundings and under tough environmental conditions where most other cereals fail. The crop is preferred by both farmers and consumers due to its beneficial traits associated with its agronomy and utilization[2]. Teff is a staple food in Ethiopia, consisting of two-thirds of their cereal diet and is mainly used to make human food. Teff can also be combined with other baking flours to produce baked products, such as muffins and cookies. Teff is very nutritious and is an important part of Ethiopia's cultural heritage and national identity.

The northern Ethiopian highlands, where it was domesticated, are the region's center of origin and diversity for the ancient tropical cereal teff (Eragrostis teff)[3]. Teff is a member of the genus Eragrostis in the grass family Poecae (Gramineae). Between 4000 and 1000 BC, it is thought that Ethiopia is where teff first appeared. Because it sells for the most money per pound of grain when compared to other cereals, it is farmed in Ethiopia for both local consumption and the market[4].

Since it is so readily lost if dropped, the word "teff" literally translates as "the lost seed" in Amharic. Teff is a warm-season annual cereal crop that can grow in low-fertility, drought-stressed, and other types of environmental circumstances. 15 kg/ha of seed should be shown at a planting depth of 0.6 to 1.3 cm. It germinates quickly and sprouts within 3 to 7 days[4]. The Ethiopian farmers harvest Teff hand when the vegetative part of the plant turns yellow. They use Oxen to trample the grass to separate the seed from the rest of the plant[5]. Two thirds of the population in Ethiopia consumes teff as a staple food[6]. In Ethiopia, it is believed that it feeds millions of people and is crucial for at least 60 million rural farmers, processors, and consumers[7]. It is typically used to make "Injera," a thin, porous, sour-tasting bread that resembles a pancake and is generally eaten with Wot[4]. Although Teff is farmed for its grain, the straw is also used to build homes in both rural and urban areas, to reinforce mud or plaster, and as feed for cattle[8]. Teff grain is widely distributed throughout the majority of the nation and is primarily grown under a variety of agro-ecological conditions at elevations between 1800 and 2100 meters above sea level. Amhara (east and west Gojam) and Oromia (east and west shewa) are the principal Teff-producing regions[9]. More than 10% of the country's yearly teff production is produced in East Gojam, making it the leading zone[10]. There are many different Teff kinds in Ethiopia, but the three main types are white, red, and mixed (sergegna)[11]. Red Teff is less frequently used to make Ethiopian bread (Injera) because it is less soft, porous, or sponge-like, and because of historic biases that ignore the distinctive qualities.

Consumers of Red Teff have increased hemoglobin levels in their blood and do not readily get anemia. Moreover, it aids in preventing anemia brought on by pregnancy. This is caused by the makeup of the minerals in teff, particularly iron. The body contains minerals, which are inorganic chemicals that work with enzymes, hormones, vitamins, and other components[12]. No mineral can function on its own; nevertheless, the bioavailability of other minerals can be affected by the presence or lack of other minerals. The examination of critical elements in Teff is required in order to establish recent data on its essential mineral composition.

Teff is made up of 3% fat, 80% complex carbohydrates, and 11% protein. It is a top supplier of vital amino acids, particularly lysine, which is frequently lacking in grain-based foods[13, 14]. Moreover, it has a little lower lysine content than rice or oats but greater than barley, millet, and wheat. In addition to being a great source of fiber and iron, teff also has significant levels of calcium, potassium, and other vital minerals that are present in comparable amounts in other grains[15, 16]. Studies already conducted[17, 18] noted that Teff flours contain higher concentrations of some critical elements (Ca, Fe, and Zn) than do other common grains. However, there is lack of reports on experiments done to measure the content of essential and non-essential metals in various Teff varieties in Yilmana Densa (Adet) Woreda in various kebeles. The aim of this study was to determine the level of essential and non-essential elements in various Teff samples cultivated in two kebeles (Fetilo Kuskuam and Kililt) in Yilmana Densa (Adet) Woreda, West Gojam Zone, Ethiopia, using FAAS.

1.2. Statement of the problem

Teff is widely acknowledged as an extremely nutritious diet, despite some controversy regarding its nutritional value[18, 19]. Some authors have assessed the content of Fe to be high, while others have indicated that this high level is due to the dust and filth that adhere to the grain[20]. But other research has shown that the Fe concentration of teff varies geographically, may be as a result of contaminated soil. A survey of the literature revealed that various methods were used at various times to determine the mineral content of teff flour. Yet, there is disagreement and divergence in the results published in the various literatures; this may be the result of sampling error. Teff flour samples were primarily obtained from commercially available mill houses, which may have increased the samples' levels of contamination.

In addition to other grains grown there, teff is predominantly grown in the chosen study areas of Yilmana Densa (Adet) woreda and is used as the primary food source. Teff samples from several regions of Ethiopia were recently revealed to contain high concentrations of critical metals. However, the amount of essential and non-essential ingredients in teff that can be grown in Yilmana Densa (Adet) Woreda, Fetlo Kuskaum and Kililt kebeles (Locality) is not reported. Now a days many people suffer from anemia which is the deficiency disease of iron, therefore this study determine the iron content of the teff samples and gives an insight for users. Due to lack of brand, the farmers that live in that area also sell their product in small price.

There for this study investigates the level of (Zn, Cu, Fe, Mn, Ni, Co, Pb and Cr) in teff grown in Yilmana Densa (Adet) Woreda by using FAAS.

1.3. Objective of the Study

1.3.1. General objective

The main goal of this study was investigating the levels of Zn, Cu, Fe, Mn, Co, Ni, Cr and Pb, in red, white and mixed teff samples collected from two kebeles (Fetlo Kuskaum and Kililt).

1.3.2. Specific objectivs

- To determine the level of Zn, Cu, Fe, Mn, Co, Ni, Cr and Pb in red, white and mixed teff samples.
- To compare the concentrations of a few essential (Zn, Cu, Fe, Mn, Co, Ni, and Cr) and non-essential (Pb) metals between the two sampling sites.
- To compare the levels of the investigated essential and non-essential metals in red, white and mixed teff samples.

1.4. Significance of the study

The results of this study will be important in formulation of mineral nutrient supplements from teff samples. Provide the levels of essential and non-essential metals contained and the consumer was informed while using the teff samples. It might provide as a starting point for additional research in the field and provide details on the concentration of the materials being researched, allowing researchers to broaden some of the efforts being made through this study.

1.5. Scope of the study

The scope of this study was determination of the level of essential and non-essential metals in red, white and mixed teff were growing in Yilmana Densa (Adet) Woreda. However two kebeles (Fetlo Kuskaum and Kililt) were selected to conduct this study for mineral analysis by using FAAS.

CHAPTER TWO

2. LITERATURE REVIEW

Teff is an Ethiopian grain crop that as long provided food for its population. The Amharic term "Tefa"[21], which meaning lost due to the small seed size and difficulty in finding it once it has been dropped, is thought to have inspired the name Teff. However, other, more reliable sources claim that it was derived from the Arabic word tahf, which was used by South Arabian Semites to refer to a related wild plant when they were in need of food security. The combined evidence suggests that the crop originated in northeastern Africa, as suggested by its limited geographic production area, language origins, and cultural and historical heritage. With the exception of oats and rice, teff has more lysine than any other grain. In addition, it has a higher Fe content than winter wheat, barley, or sorghum, as well as higher Ca, Cu, Zn, and Al concentrations[4].

Teff, love grass, annual bunch grass, and warm season are some of its common names. There are several varieties of teff, each with characteristics best suited to specific conditions. It is not in the scope of this paper to discuss the details about all the different varieties of this grain. Although there is no unifying classification of the teff type throughout the country, in general, there are three main types of teff: white, red and mixed (sergegna).

White teff is the preferred type but only grows in certain regions of Ethiopia. White teff grows only in the Highlands of Ethiopia, requires the most rigorous growing conditions, and is the most expensive form of teff.

The prestige associated with consuming white teff, as well as its more stringent growing conditions, contributes to the increased cost of white teff. The shelf life of Enjera is extended with the use of white teff[22].

Red teff is the least expensive form and the least preferred type, has the highest iron content[23]. In persons living in areas of the country where consumption of red teff is most prevalent, hemoglobin levels were found to be higher with a decreased risk of anemia related to parasitic infection[23]. According to the studies, due to the increased health benefits associated with high iron contents in red teff, there is more acceptance of this grain in the society. Today in Ethiopia, red teff is becoming more popular related to its increased iron content. However, few researchers indicate that the level of iron in the teff is related to the threshing of the grain on the soil[23].

Teff grains are still a significant staple food in Ethiopia and are thought to have originated there between 4000 and 1000 B.C. The teff plant's straw is soft and quick to dry; making it ideal for a variety of uses such clay pots for the home and building materials[14]. Teff grows well in warm climates with temperatures between 10 and 27 °C and altitudes between (1000-2100m). It can, however, endure extreme conditions including soil that is saturated with water or dryness.

Teff has higher mineral concentrations than wheat, barley, or sorghum. Teff flour has higher levels of Fe, Ca, and Zn than other common cereals, according to previously published studies employing UV-Visible and FAAS to measure the levels of several critical metals in teff flours.

However, there is conflicting and inconsistent evidence in the literature[8] on the major, minor, and trace element contents in teff flours. According to the Ethiopian Central Statistical Authority, it is grown on 2.7 million hectares and is thought to account for 16% of the output of cereal grains. It is the main meal in the country's central, northern, and western regions[24].

Teff is a highly esteemed cereal crop, and in some regions of the country, a family without teff as a staple diet is regarded as being destitute. The fundamental cause of this mindset is the society's long-standing development of teff's socioeconomic, cultural, and historical significance. In the north and central parts of Ethiopia, injera made from any crop is the most often consumed food. As other grains like wheat and barley lack the necessary organoleptic properties, many people dislike injera cooked from them attributes of injera. When they have enough other grains, families frequently sell them for less money and then purchase teff. More Ethiopians than any other ethnic group are familiar with the flavor of teff injera. Teff is believed to have a superior source of fiber, iron, and calcium compared to other cereal grains, making it the most valued grain in Ethiopia in terms of nutrition[24].

The study by the Ethiopian Institute Biodiversity Conservation Laboratory from 114 teff variants collected from Ethiopia indicated that teff grains contain average of 9-15 % protein, 2-4 % fat, 2- 4 % fiber, 68-74 % carbohydrate, 10-13 % of moisture and ash content.

2.1. Origin of teff

Between 4000 and 1000 B.C., Ethiopia is where teff was first domesticated [13]. It is one of the African grain crops that were domesticated by Africans, who harvested the seed grains of native wild grasses for food and selected the traits that best fit their preferences, farming methods, and growing environments. Teff seeds were reportedly discovered in the pyramid of Dashur (3359 B.C.) and the ancient Jewish settlement of Ramses in Egypt, according to Tadesse's report (p. 72). (1300 B.C). Although Costanza[25] hypothesized that the word teff might have come from the Semitic thaf used in Yemen to refer to a crop that was collected from the wild. But another source[26] offered the following definitions of "thaf" from an Arabic dictionary: The Yemeni plant known as taf' produces grains that are consumed during times of hunger and resemble those of red mustard.

Figure 1: Teff plant captured from the sampling sites

2.2. The importance of Teff

Two-thirds of the daily protein requirements for the Ethiopian people are met by teff. Additionally, it provides estimates for 31% of the cereals and nearly 26% of the annual crops. It is frequently crushed into flour to form the well-known thin, airy Ethiopian bread known as "Injera"[27]. A significant staple dish for many Ethiopians is injera. Also, it is used to make cakes, delicious dry unleavened bread, the native alcoholic beverages Tela and Katikalla, porridge (Muk), and other baked goods[28].

Teff grain nutrients provide celiac patients a possible alternative to wheat-based foods. Its straw is most frequently utilized as mud-based building material and as animal feed[29]. It is the most nutrient of all the grains planted in Ethiopia, and for farmers, it yields more value than any other cereals. The majority of Ethiopians enjoy eating teff, although only middle- and high-class urban dwellers have access to this grain. In urban regions, it contributes roughly 600 kcal per day, compared to 200 kcal in rural areas[30]. Teff's alleged antioxidant abilities imply that health food products may be made using it. Teff is a grain that has recently gained appeal in the food sector as a nutrient-rich baby food ingredient and a naturally gluten-free substitute for wheat products [31].

2.3. Production and distribution of teff

Ethiopia is regarded as the country's origin, where teff has been grown for at least 2000 years. The Amhara and Oromia regions are where it is mostly farmed, accounting for 86% and 88.85%, respectively, of the total cultivated area and output. In Tigray and the Southern Nations Nationalities and Peoples (SNNP) region, less teff is also produced[32]. According to CSA 2013 data, 19 zones in the Tigray, Amhara, and Oromia regions account for more than 83% of the nation's teff output. Particularly well-known teff-producing regions in the nation include East and West Gojam of Amhara and East and West shewa of Oromia[32]. Teff grains can range in color from ivory to dark reddish-brown purple, depending on the variety. Depending on consumer preferences, white teff rises to the top of the price list, whereas red Teff, which has a higher nutritional value, is accepted by Ethiopians who value their health[33]. Teff offers a lot of benefits and requires large-scale production, but its productivity has fallen short of its potential. Teff's average national productivity is currently 1.664 t/ha, which is relatively low compared to other cereal grains farmed in Ethiopia. This is because inadequate soil fertility and other variables are yield-limiting.

2.4. Major climatic conditions for teff grown in Ethiopia

Teff can grow in a variety of locations and is now grown under various agroecological circumstances. It can be grown at any altitude between sea level to 2800 meters above it, depending on the rainfall, temperature, and soil conditions. But, the ideal environment for growing great teff is between 1800 and 2100 meters above sea level, with temperatures between 10 and 27 degrees Celsius and yearly precipitation of 750 to 850 millimeters.

This almost exactly matches the heights in Ethiopia's highland regions[4]. One of the reasons teff is favored over other grain crops like maize or barley is its capacity to perform well on both water-logged Vertisols in the highlands and water-stressed areas in the semi-arid regions across the entire country. The plants also germinate quickly and are suited to a variety of situations, including waterlogged soil and drought stress. Generally speaking, teff is less susceptible to biotic stressors than the majority of other Ethiopian cereal crops. This crop is dependable and minimal risk[34].

2.5. Market conditions of teff in Ethiopia

Teff is a gluten-free food crop that is mostly farmed by small holder farmers, which has led to a great deal of interest in it on the global market. Due to its high demand and high market value, teff farming generates more profits for farmers than other types of staple crops[13].

Depending on the kind of teff, prices have climbed by between 30 and 36% during the past ten years (red, white or mixed). However compared to market pricing in 2008, the cost of teff rose sharply in the 2009 crop year, jumping up to 35%. In example, the price differential between teff and maize has grown significantly. Teff production-friendly weather, the availability of manpower for peak agricultural operations, and the annual application of better agricultural technologies are all factors that affect the volume of surplus teff that is marketed[35].

Teff is spreading to other locations because of the strong demand for its use. While the majority of the grains pass through Addis Ababa, according to the FAO, some urban consumption hubs, such Mekele and Desie, receive their supplies directly from the production areas. Adama and Nazeret supply other urban areas including Harar and Dire Dawa (East Shewa). For customers, other grains are a poor substitute for teff due to its taste and preference. These factors contribute to Ethiopia's consistently high teff demand, which is less sensitive to price fluctuations than those of other grain crops[35].

Teff started to be exported to the Middle East, North America, and Europe to meet the demand of Ethiopians living abroad as a result of increased globalization and labor migration. Teff export levels have significantly decreased since 2006 as a result of the 2007 restriction on grain exports. However, the cost of teff has remained relatively high due to high demand brought on by rising incomes (as a result of the booming service and industrial sectors, particularly the construction sector), high rates of urbanization, a rapidly growing demand for fresh injera exported, and smuggling into neighboring countries[36].

2.6. Nutritional compositions of teff

The primary component of teff caryopsis, as well as various teff kernel tissues, is carbohydrates. Starch, which makes up the majority of the carbohydrates and accounts for 73% of the dry weight of the kernel, is mostly concentrated in the endosperm. As there can be no fractionation during the milling process because of the small size of teflon grains, the higher fiber content in teflon results from the fact that it is always ingested in the full grain form (bran and germ combined)[33].

Teff has a notably high fiber content of 3% (dry basis), which is more than that of most other cereals including wheat (2%), rye (1.5%), rice (0.6-1.0%), and sorghum (0.6%). Teff grains are nutritionally dense, containing 7.3% starch in the whole kernel that is then stored as polygonal starch granules in the endosperm of the grain, 9.4-13.3% protein, and an excellent balance of essential amino acids, primarily leucine, valine, proline, alanine, and glutamic and aspartic acids. 2.6-3.0% ash, 2.0-3.1% lipid, and richer in Fe, Ca, Zn, and Mg than other cereal grains, according to studies[37].

According to Bultose (2007), teff has greater starch content than most cereals and has an endosperm that is predominately made up of starch (73%). The crude fat percentage of teff is in the range of 2.0-3.05 with mean values of 2.3%, and the grain protein content of 13 teff types ranged from 8.7-11.15% with mean values of 10.4%. Teff has a high protein level that includes all eight necessary amino acids and is superior to wheat or barley in terms of lysine content, along with a high carbohydrate and fiber content[7].

Teff contains less protein than quinoa and is comparable in protein content to other common grains such barley, wheat, maize, and pearl millet. Teff grains contain a high percentage of unsaturated fatty acids (72.46%), of which 30.91% were polyunsaturated and 20.06% were saturated fatty acid[7]. Niacin and Vitamins A and C are present in significant amounts in teff, and their quantity is typically boosted by the yeast fermentation process used to make "injera".

2.7. Mineral contents of teff

Minerals, which serve crucial roles in the body but cannot be created by living things like carbohydrates, lipids, or proteins, are inorganic substances. Inadequate intake or decreased bioavailability can lead to physiological imbalances and impairment of essential activities. Calcium, phosphorus, iron, potassium, sulfur, sodium, magnesium, zinc, copper, selenium, and chromium are a few of the most well-known[38]. Teff has a higher mineral concentration than wheat, barley, and sorghum. Fermented teff dishes are also a mainstay in the production areas. Fermentation is known to contribute to high Fe availability in diets by destroying phytic acid. This explains why anemia is uncommon in Ethiopia's highlands, where teff is the country's primary grain crop[18].

Teff eaters have higher hemoglobin levels in their blood than non-teff eaters, and they do not experience hookworm anemia despite being infested, according to studies; hookworm anemia manifests in non-teff eaters who are infested with hookworm. Teff consumption in Ethiopia appears to be correlated with a lack of anemia, which is thought to be caused by the grain's high iron concentration. Moreover, malaria is frequently detected in groups with lower hemoglobin levels, per the same research[39].

High quantities of Ca, P, Cu, Zn, and Mg are also present in teff. Teff has a much greater concentration of calcium (147 mg/100 g) than other grains such as maize (16 mg/100 g), sorghum (5.8 mg/100 g), wheat (39.5 mg/100 g), and rice (23 mg/100 g). Teff (6.7mg/100g) has a greater zinc concentration than sorghum (1.7mg/100g), wheat (1.7mg/100g), and rice (2.2mg/100g)[28].

2.8 Physiological role of some minerals

2.8.1. Iron (Fe)

Iron is a necessary mineral for humans since it is a component of hemoglobin, myoglobin, and several enzymes. Several essential bodily processes rely on iron. It functions as an integral component of crucial enzyme systems in diverse tissues, as a transport medium for electrons inside cells, and as a carrier of oxygen to the tissues from the lungs through red blood cell hemoglobin[40]. Teff consumption in Ethiopia appears to be correlated with a lack of anemia, which is thought to be caused by the grain's high iron concentration. Lower hemoglobin categories are more likely to get malaria.

2.8.2. Copper (Cu)

A vital trace element for both humans and animals, copper serves a variety of purposes in plants. It is necessary for the synthesis of certain enzymes, including tyrosine, erythrocytes, and hemoglobin. The development of elastic tissue and bones is also influenced by copper[41]. A lack of copper causes anemia due to improper iron metabolism, skeletal defects, immune and cardiovascular system problems, particularly in babies, defects in the pigmentation and structure of hair or wool, infertility, and diminished arterial elasticity.

Teff was found to have 8.1 mg/kg of copper, according to the USDA, which is comparable to the copper concentration of Bolivian-grown teff. In modest amounts, copper is necessary for the human body since it is a component of redox enzymes. For both genders, ages 31 to 50, the RDA for copper is 900 g/day[42]. Wilson's illnesses, nervous system disorders, and vomiting can all be brought on by an excessive copper intake[43].

2.8.3. Zinc (Zn)

Many enzymes involved in the synthesis and breakdown of lipids, proteins, nucleic acids, and carbohydrates as well as the metabolism of other micronutrients all require zinc as an essential component. Growth retardation, delayed sexual and bone maturation, eye and skin lesions, diarrhea, baldness, reduced appetite, increased susceptibility to infections caused by immune system deficiencies, and the emergence of behavioral disorders are all symptoms of severe zinc deficiency in humans[40].

According to Abebe et al., 2007, there are roughly 40.2 mg/kg of zinc in brown teff and 28.6 mg/kg in white teff. Whereas Nevin and Navruz-Varli reported in 2016. Teff (6.7mg/100g) has a greater zinc concentration than sorghum (1.7mg/100g), wheat (1.7mg/100g), and rice (2.2mg/100g). The RDA is 8 mg/day for females and 11 mg/day for males aged 31 to 50[44].

2.8.4. Manganese (Mn)

Both plants and animals require the element manganese. It is vital for crucial enzyme processes and proper bone metabolism. Moreover, it supports healthy thyroid, brain, and nerve function. The USDA estimated the manganese content of teff to be approximately 92.4 mg/kg in 2014. Many enzymes may use manganese as an enzyme activator. Deficiency is unlikely if a healthy diet is maintained because it is widely distributed in food[45]. The AI values for manganese for males and females aged 31 to 50 have been defined by the IOM, 2001, at 2.3 and 1.8mg/day, respectively. Poor reproductive performance, growth retardation, congenital deformities in the progeny, improper bone and cartilage formation, alterations in carbohydrate and lipid metabolism, and decreased glucose tolerance are all symptoms of Mn deficiency. Moreover, it impedes bone development[46]. Increased slowness and clumsiness of body motions might result with excessive Mn exposure, along with mental and emotional disturbances.

2.8.5. Chromium (Cr)

Trivalent chromium is known to increase insulin activity and take role in glucose metabolism. Hexavalent chromium is poisonous, but with both acute (short-term) and chronic (long-term) inhalation exposures to chromium (VI) poisoning, the respiratory tract is the primary target organ [44]. Shortness of breath, coughing, and wheezing have been observed in cases of acute exposure to chromium(VI), but chronic exposure has been linked to bronchitis, pneumonia, septal perforations and ulcerations, impaired pulmonary function, and other respiratory consequences. Chromium (VI) inhaled is a human carcinogen that raises the risk of developing lung cancer. According to Kabata-pendias (2011), cereals can contain anywhere between 10 and 90 g/kg of chromium. The AIs are 35 and 25 g/day for men and women aged 31 to 50, respectively[44].

2.8.6. Nickel (Ni)

Nickel is thought to participate in physiological processes as a co-factor in the intestinal absorption of iron. Only when dietary iron was in the insoluble ferric form did nickel boost the absorption of iron from the diet in iron insufficiency[47]. Contact with nickel compounds can have a number of harmful impacts on human health, including contact dermatitis, lung fibrosis, kidney and cardiovascular illness, and respiratory tract cancer.

Ni has a number of acute health consequences that might cause nausea, vomiting, abdominal pain, diarrhea, vision disturbances, headaches, giddiness, and coughing. A cutaneous rash at the point of contact is the reaction to nickel exposure that occurs most frequently[48].

2.8.7. Cobalt (Co)

Cobalt is only known to be necessary for animals in the form of vitamin B12, which contains 4% cobalt by weight. Thus, a cobalt shortage is actually a vitamin B12 deficiency. Loss of appetite, emaciation, weakness, anemia, and decreased production are all signs of cobalt deficiency. A cardiomyopathy with a high mortality risk is caused by excess cobalt. Cobalt's RDA is 0.3 milligrams per day. Cobalt must be consumed for it to have biological value[5].

2.8.8. Lead (Pb)

Lead causes blood problems and is bad for the brain system. The brain, heart, kidney, liver, neurological system, and pancreas are all affected by lead toxicity. Abdominal discomfort, anemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, exhaustion, headaches, and hypertension are just a few of the indications and symptoms that it may produce. Moreover, it lowers children's IQ[49].

2.8.9. Cadmium (Cd)

Due to its high toxicity, wide solubility in soil and water, and ease of accumulating in the roots of most plant tissues, cadmium has been regarded as a very major contaminant affecting all living forms despite having no recognized nutritional benefit. Over exposure to Cd may result in cancer, kidney, lung, hepatic, skeletal, and reproductive problems. Acute gastrointestinal consequences from eating food contaminated with cadmium include vomiting and diarrhea[50, 51].

Table 1: Health benefits/main functions and sources of essential metals

Mineral	Health benefit	Sources	Reference
Ca	For developing and maintaining healthy bones and teeth	Teff, milk and dairy products, a few green vegitables,dried fruits and drinking water(including mineral water)	11
Fe	For red blood cells formation and required for oxygen transport throughout the body	Seafood,meat, poultry or organ food like liver, vegetables such as beans and peas nut,seeds	10
Zn	For digestion, metabolism, reproduction and wound healing	Wheat, brown rice,oats,lentils.soybeans, dried peas, black –eyed peas,limabeans,walnuts,peanuts,cashews,brazil nuts,cheeses,any kind of liver, animal flesh such as beaf,lamb,chicken,turkey, and various fish and seafood	12
Mn	Support brain function and required for blood sugar regulation	Surface water and groundwater, foods,tea, green vegetables, nuts, bread and other cereals	12
Cu	To normal red blood cells formation and connective tissue formation	Wheat, barley, sunflower seeds,almonds, pecans,walnuts, peanut, cashews, prunes, raisins apricots, various dried beans, mushrooms, chicken and most fish	12

2.9. Sample Decomposition Techniques

By removing interfering substances from the matrix and retrieving the element in a homogenous and accessible matrix, sample decomposition is beneficial for converting all the species in which a given element is present in such a way that it becomes present in one. The final determination's purpose and variables such the matrix composition, elemental contents, potential interferences, danger of losses and contamination, practicality, and potential safety risks in the lab should all be taken into consideration when choosing a breakdown procedure[52]. There are several types of breakdown techniques: 1. Dry ashing, 2. Wet digestion, 3. Micro wave digestion.

2.9.1. Dry ashing techniques

The impact of organic components on the determination of mineral elements in food materials is eliminated or significantly reduced by dry ashing or oxidation. In a muffle furnace, it involves igniting organic compounds in air at atmospheric pressure and moderately high temperatures (450–550°C).

The use of ashing aids may be necessary to complete the composition of organic matter in tissues that are high in carbohydrate and lipid content. In the presence of air, organic molecules burn and water and other volatile components vaporize. An appropriate acid is used to dissolve the resulting ash residue.

2.9.2. Wet-digestion techniques

Heat and mineral acid(s) are both used in wet digesting processes. H_2SO_4, HNO_3, and $HClO_4$ have all been employed in this technique, either together or separately. Furthermore, hydrogen peroxide is utilized to quicken reactions and guarantee thorough digestion. Because to the risk of explosion, HClO4 is no longer used in the majority of laboratories. Wet digestion may be done in open vessels, tubes, hot plates, aluminum heating blocks, closed vessels under raised pressure (digestion bombs), or closed vessels heated by thermal or microwave energy. The microwave-assisted digesting approach is appealing, particularly for tiny samples[53, 54].

2.9.3 Microwave-assisted digestion

One of the main benefits of MW-assisted digestion is the significantly reduced reaction time (minutes) needed, direct heating of samples and reagents, decreased need for aggressive reagents, low contamination, and absence of volatile element loss. For MW-microwave (MV)-assisted digestion using nitric acid or a combination of nitric and hydrochloric acids alone or with assisted digestion, two alternative systems are available: pressurized closed-vessel systems and open focused-MW systems that operate under atmospheric pressure. The use of microwave-assisted digestion in a closed vessel under pressure has grown in popularity as a quick and easy method of dissolving materials that reduces acid usage, sample contamination risk, and volatile element loss.

One of its drawbacks is the length of time, which can range from minutes to hours that the containers must cool before they can be opened. Focused MW radiation has several benefits, including safety, adaptability, control over the amount of microwave energy emitted into the sample, and the ability to add solutions on a schedule while the sample is being digested.

Open-vessel digestion cannot entirely rule out the loss of volatile elements, and results for low-level elements may be impacted by the large amount of reagents utilized, increasing the likelihood of sample contamination. Using vapor-phase acid digestion, which has shown to be very successful in reducing the residual carbon content, can help to reduce this risk. A common method for dissolving samples is the addition of hydrogen peroxide[54].

2.10. Analysis of Mineral Nutrients

Many analytical techniques are used to identify the metals in samples, with the choice frequently based on the level of sensitivity and precision needed. Both macro and micro elements can be determined by various spectroscopic or chromatographic methods, such as atomic absorbance spectrometry using flame(FAAS) or graphite furnace(GFAAS) atomization, atomic\semission spectrometry(AES), inductively coupled plasma optical emission spectrometry(ICPOES), inductively coupled plasma mass spectrometry(ICPMS), x-ray fluorescence(XRF) and ion chromatography(IC). In this study, the mineral nutrients in samples of teff sample were determined using FAAS. Before analysis, all of these techniques including the FAAS require sample pretreatment. One of these sample pre-treatment techniques involves the breakdown or decomposition of the sample matrix. The goal of sample decomposition is to transform all the species in which a specific element is present into a defined form, remove interfering substances from the matrix, and extract the element in a homogenous and accessible matrix[52].

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CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the study area

The two study areas are located in Amhara Regional state in West Gojam zone, Yilmana Densa Woreda. The Woreda is situated in north- western highlands of Ethiopia. Yilmana Densa (Adet) is one of the woreda in the Amhara Regional state of Ethiopia, part of West Gojam Zone. Yilmana Densa District has 35 rural administrative kebeles, 4 sub municipal cities, 5 city administration kebeles. Yilmana Densa (Adet) is bordered on the South by Kuarit woreda and Sekela woreda, on the north by Bahir Dar Zuria woreda, on the east by Gonji kolela woreda and south Gonder zone, on the west by the Mecha wereda. It is 450 kilometers far from Addis Ababa along the Addiss Ababa- Bahir Dar road at a distance of 42 kilometers south of Bahir Dar. The topography of Yilmana Densa District consists of various land forms, mountains (20%), Plains (56 %), valleys and caves (8 %), swamp areas (0.05 %) and others (15.95 %). The climatic condition of the district is categorized as 64% Weyna Dega (sub-tropical), 24% Dega (temperate).12% kolama (lowland). The annual average temperature of the Yilmana Densa District ranges between 15 °C up to 27°C and it receives about 1200-1600 mm mean annual rainfall. This shows that it is entirely located in Sub-tropical climatic zones (Yilmana Densa Woreda Agricultural and Rural Development office and Yilmana Densa Branch Meteorology Agency, 2021). Based on Yilmana Densa Woreda Agriculture and Rural Development Office (2021), the total area of the district is 94,180 hectares. The total population of Yilmana Densa District was estimated to be 295,574, of which 139,241 are males and 156,333 are females. Among the total of population 25,060 males and 26,151 females are urban residents and 114,181 males and 130,181 females are rural inhabitants (YDWCSO, 2020). Fetlo Kuskuam and Kililt kebeles are found in the direction of east from Adet town. Adet is located in the south of Bahir Dar in the West Gojjam Zone of the Amhara Region.This town has a latitude and longitude of 11°16′N 37°29′E with an altitude of 2,216 meters above sea level. It is the largest settlement in Yilmana Densa woreda. The most teff-growing kebeles in the woreda, Fetlo kuskaum and Kililt, were chosen as the two sampling kebeles.

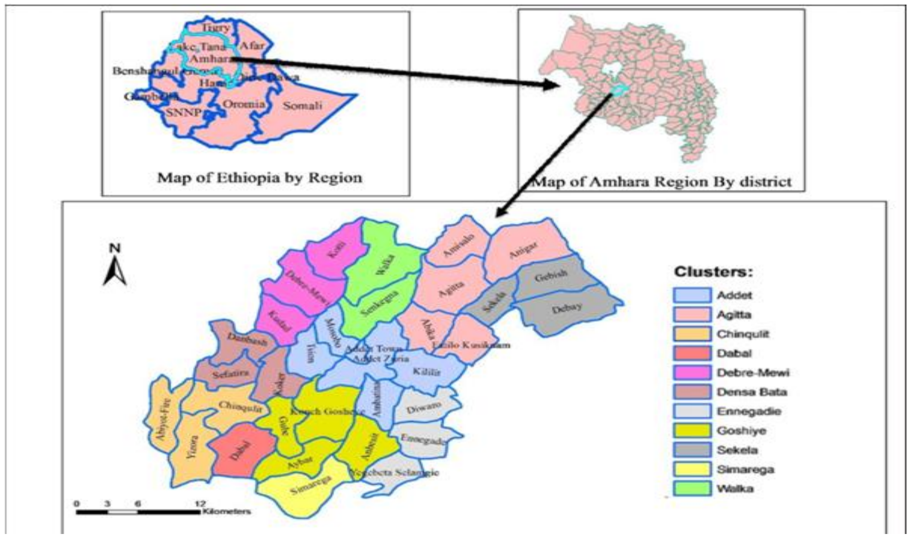


Figure 2: Map of Yilmana Densa District by Kebele, in Amhara region, Northwest of Ethiopia (Amhara Urban planning institute, AUPI 2016/2017)

3.2. Chemicals and reagents

The following analytical grade reagents were used as received. HNO_3 (69–70%; Superior Enterprises Cantt, Germany) and H_2O_2 (30%; Scharlau, Germany). Stock standard solutions (Buck Scientific purographics calibration standards, USA) containing 1000 mg/L of the metals Cr, Mn, Ni, Cu, Zn, Pb and Co from which 10 mg/L of intermediate standard obtained were used for preparation of calibration standards of each metal. 1,10 phenanthroline hydrate(99.8% ,Sigma Aldrich, Germany), sodium acetate Trihydrate (99%, Sigma Aldrich, Germany), ferrous ammonium sulfate hex hydrate (98%,Sigma Aldrich, Germany) were used for the determination of Iron metal in the teff sample. Throughout the experiment deionized water was used for sample preparation, dilution and rinsing apparatus before analysis.

3.3. Instrument

The following instruments were used electrical blending device (Mettler Toledo Model at 250 and Switzerland), Flame atomic absorption spectroscopy (Buck Scientific, Model 210VGP AAS, USA) equipped with deuterium background corrector and air-acetylene flame atomizer was used for analysis of the selected metals (Cr, Co, Mn, Ni, Cu, Zn and Pb) in teff sample and Single beam spectrophotometer (Model Cecil Instruments Cambridge England, serial No.121-789) and glass cuvettes were instrument used to determine iron. A ceramic mortar and pestle (USA) were used for grinding and homogenizing the sample. A digital analytical balance (with 0.1 mg precision) was used for weighing the samples.

3.4. Experimental Procedure

3.4.1. Cleaning apparatus

Volumetric flasks, measuring cylinders, and digesting beakers were among the equipment that were washed in detergent and tap water before being rinsed with distilled water. Following a 24-hour soak in 1% (v/v) nitric acid, these instruments were three times washed with distilled water and were stored in a dust-free environment until the analysis began.

3.4.2. Sampling and sample preparation

Each of the two chosen sampling kebeles in Yilmana Densa woreda provided a farmer with 300g of white, mixed and red teff grain samples was collected from two randomly selected farmers from each kebele using plastic bags. Each sample was then marked and put in polyethylene plastic bags for delivery to the lab for additional processing. Light and heavy contaminants were removed from the samples by sieving, sifting, and winnowing prior to washing. The samples were cleaned and maintained in a 70°C oven for 12 hours. The dried materials was homogenized, grinded, and sieved after being passed through a mortar and pestle. Following that teff samples were stored in, plastic bags Figure 3. Until analysis.

Figure 3: Image of Teff sample preparation in leveled bags

Figure 4: Image of oven and teff sample

Figure 5: The grinded sample of Teff

3.4.3. Digestion of Teff samples

Optimized wet digestion procedure was employed for mineralization of the teff samples to obtain a clear solution. Optimum condition was obtained by making different trials using various volumes of solvents, times and temperatures of digestion. The different conditions taken and tried are shown in **Table 2,** teff sample was digested According to the procedure described in the literature with slight modification[55]. 0.5g of the powdered and homogenized teff sample was added into a 100ml beaker, to which 8ml of HNO_3 and 2ml of H_2O_2 were then added. On a heating mantle Figure 6, the mixture was digested two and half hours at a temperature of 180°C. Following sample digestion, the digested solution was allowed to cool and 20 ml of distilled water was added, and 50 ml of volumetric flask were then filled to the mark with distilled water. The digested samples were then put in the refrigerator until analysis.

Figure 6: Digestion of teff sample

Table 2: Optimization of digestion procedure

Trial	Reagent volume Ml		Total	Temperature ^{o}C	Time (h)	Observation
	HNO_3	H_2O_2				
1	4	1	5	180	2:30	Red brown
2	5	1	6	180	2:30	Yellow
3	6	1	7	180	2:30	Slightly colorless
4	**8**	**2**	**10**	**180**	**3:00**	**Colorless**
5	8	2	10	180	0:30	Cloudy suspension
6	8	2	10	180	1:30	Yellow
7	8	2	10	180	2:30	Colorless
8	**8**	**2**	**10**	**180**	**3:00**	**Colorless**
9	8	2	10	60	2:30	Red brown
10	8	2	10	120	2:30	Yellow
11	8	2	10	180	2:30	Slightly colorless
12	8	2	10	240	2:30	Colorless

3.5 Operating Conditions of FAAS for Analysis of Metals

In this study a total of eight metals for each teff were analyzed using FAAS with external calibration curve after the parameters such as burner and lamp alignment, slit width and wavelength adjustment were optimized for maximum signal intensity of the instrument. For each metal, the respective hollow cathode lamp was inserted in to the atomic absorption spectrophotometer, and the solution was successively aspirated into the flame. Hallow cathode lamp for each metal, operated at the manufacturer's recommended conditions, and were used at its corresponding primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. Seven elements (Pb, Cr, Mn, Ni, Cu, Zn and Co,) were analyzed by absorption mode of the instrument. The operating conditions of the instrument employed for each analyte are shown in **Table3.**

Table 3: Instrumental Operating Conditions for Determination of Selected Metals in teff Sample Using FAAS

Metals	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Energy (eV)	Instrumental detection limit (mg/L)
Cr	357.9	0.7	2.0	2.712	0.040
Ni	341.5	0.2	7.0	2.624	0.020
Cu	324.7	0.7	1.5	3.938	0.005
Zn	213.9	0.7	2.0	3.237	0.005
Pb	283.2	0.7	2.0	2.874	0.040
Co	240.7	0.2	4.5	3.106	0.050
Mn	279.5	0.7	3.0	3.913	0.030

3.5.1 Instrument Calibration and Measurement of Selected Metals Concentration

Determination of trace metals concentration was done by using FAAS (Buck Scientific Model 210VGP) in Hawassa University Chemistry Laboratory. Intermediate standard solutions (10 mg/L) of metals of interest were prepared from the 1000 mg/L standard stock solutions. These solutions were diluted to the desired concentrations to calibrate the instrument (A 10 ppm multi-element solution containing Cr, Zn, Mn, Co, Ni, Pb and Cu was utilized to prepare elemental calibration solutions).

This multi-element solution was diluted with 2% nitric acid to obtain working standards for each metal of interest).These intermediate standards were diluted with deionized water to obtain four working standards of each metal. After shaking and homogenizing the solutions, the selected metals were analyzed after the instrumental operating conditions were optimized for maximum signal intensity of the instrument. Calibration curves for Cr, Mn, Co, Ni, Zn, Pb and Cu were obtained by using suitable standard solutions prepared from stock solutions. Calibration curves for each selected metal was set to ensure the accuracy of the instrument and to confirm that the results of determination were true and reliable. The quality of results obtained for heavy metal analysis using FAAS are seriously affected by the calibration and standard solution preparation procedures. Calibration standards for the elements analyzed were prepared in concentration range expected for the analyte in the samples analyzed. In addition, the calibration standards were prepared by taking into consideration the optimum working ranges of the elements. The correlation coefficient (R^2) values that are closer to the absolute value of 1 indicate that there is a strong relationship between the variables being correlated whereas values closer to 0 indicate that there is no linear relationship. As shown in Table 4, the correlation coefficients of metals were found to be from 0.9938-0.99989, which indicate strong relationship. The correlation coefficients of the elements were determined using prepared standards versus their corresponding absorbance. The prepared standard concentration and the corresponding correlation coefficients of the calibration curve for each metal were presented in Table 4. After making sure that the instrument was properly calibrated; concentration of metals in each teff sample was measured. Each of the sample solutions was aspirated into the FAAS instrument then the concentrations of each metal and emission/intensity values of the metals were recorded. Triplicate determinations were carried out for each sample. The same analytical procedure was engaged for the determination of elements in a total of six digested blank solutions for teff samples.

Table 4: Concentrations of the Standard Solutions Used to Establish Calibration Graphs for the Determination of Metals in teff Samples and their Corresponding Correlation Coefficients

S.no	Analyte (metal)	Intermediate Standard concentration in mg/L	Standard concentration in mg/L	Correlation coefficient of calibration curve (R^2)	Calibration Curve Equation
1	Cr	10	0.05, 0.5, 1, 2	0.99981	Y=0.07669C+6.33575
2	Cu	10	0.01, 0.01, 0.5, 1	0.99913	Y=0.16342C-3.99258
3	Ni	10	0.05, 0.1, 0.5, 1	0.99989	Y=0.02719C+2.62289
4	Zn	10	0.05, 0.1, 0.5, 1	0.99948	Y=0.33854C-3.57426
5	Pb	10	0.05, 0.1, 0.5, 1	0.99973	Y=0.02756C-2.09787
6	Mn	10	0.03, 0.1, 0.3, 0.5	0.9991	Y=0.11151C-8.5065
7	Co	10	0.05, 0.5, 1, 2	0.99823	Y=0.04285C+2.16087
8	Fe	10	0.025, 0.25,0.75,1	0.9938	Y=0.66727C+0.15619

Preparation of 1, 10-phenanthroline solution (0.1% w/v) was done by weighing 0.1 g of 1, 10-phenanthroline monohydrate and dissolving in 100 mL of deionized water. The solution was warmed at 80 ℃ to make it effective. Hydroxylamine hydrochloride solution (10% w/v) solution was prepared by weighing 10 g of Hydroxylamine hydrochloride in deionized water and dilute to 100 mL. Acetate buffer solution was prepared by dissolving 8.3 g of anhydrous sodium acetate (previously dried at 100ºC) in water, 12 ml of glacial acetic acid and dilute to 100 ml. Determination of Iron in teff samples were performed by pipetting 10 mL aliquot of wet digestion sample solution into 25 mL volumetric flask, and adding 1 ml hydroxylamine hydrochloride solution. After 5 min, add 5 ml buffer solution and 1 ml 1, 10-phenanthroline solution were added and was diluted to volume. Iron standard solution of 10 mg/L was prepared by weighing accurately about 0.007 g of ferrous ammonium sulfate hexahydrate. It was dissolved in deionized water, and the solution was transferred to a 100 ml of volumetric flask. 2 ml of concentrated hydrochloric acid was added and the solution was diluted to the mark.

The concentration of the solution used was 10 mg/L of iron. Iron standard series prepared from10 mg/L. These concentrations were 0.025, 0.25, 0.50, 0.75 and 1 mg/l. Pipette 0.0, 0.0625, 0.0625, 1.875 and 2.5 ml of Fe standard solution into a series of 25 ml volumetric flasks and add to each of them exactly 0.2 ml of conc. HCl. Dilute each of them to exactly 10 ml with double distilled water, and then add reagents in the same way as for the sample. The absorbance of the iron- phenanthroline complex was recorded by measuring the absorbance. After adjusting the wavelength at which to measure the standards and Teff samples, the absorbance of all standard concentrations were recorded by using distilled water as a reference blank. All teff samples were measured in the same way. Concentration of iron in teff samples was determined using UV visible spectrophotometer by adjusting the wavelength at 510 nm. From absorbance reading, determined Fe content present in the teff samples was taken by referring to standard curve. Triplicate determinations were carried out on each sample.

3.5.2 Method Detection Limit

Method detection limit is the smallest mass of analyte that can be distinguished from statistical variations in the blanks, which usually corresponds to the standard deviation of the blank solution times a constant or it is the amount of analyte that provides a signal equal to three times the standard deviation of the blank [2, 39]. Instrument detection limits were directly acquired from the instrument manual for each element under study. In this study, after the digestion of six blank solutions containing HNO_3 and H_2O_2 30 %, six readings were taken from FAAS and the standard deviation was calculated. The detection limits were obtained by multiplying the standard deviation of the reagent blank by three (MDL=3SD). As shown in **Table5,** the method detection limit of each element is above the instrument detection limit. The method detection limit of the metal was obtained by using the equation used in reference [51]:

$MDL = 3 \times SD$ ………………………………………………………Equation (1)here: MDL is method detection limit and SD is standard deviation of the blank reading

Table 5: Method and Instrument Detection Limits for Each Metal Analyzed

Analyte	Cu	Cr	Ni	Co	Mn	Pb	Zn	Fe
MDL (mg/kg)	0.45	0.27	0.46	0.49	0.16	0.081	0.38	0.56
IDL for selected metals(mg/kg	0.005	0.040	0.020	0.050	0.03	0.040	0.005	-

MDL = Method Detection Limit

IDL= Instrumental Detection Limit

3.5.3 Method Performance Validation

In present study due to the absence of certified reference materials for teff sample in our laboratory, the validity of the digestion procedure, precision and accuracy of FAAS were assured by spiking the concentration of teff with standard of known concentration. The spiked and non-spiked teff samples were digested following the same procedure employed in the digestion of the respective samples and analyzed in similar condition. All the spiked samples were digested in triplicate following the optimized digestion procedure of teff samples. The digested spiked samples were analyzed for their respective metals content using FAAS and UV-VIS. Then the percentage recoveries of the analyte were calculated by [51]:

$$\%\ Recovery = \frac{C(spiked) - C(non\ spiked)}{C(added)} \times 100\% \quad \text{..............................Equation (2)}$$

Where; C (spiked) is metal content of the spiked sample, C (non-spiked) metal content of non-spiked sample and C (added) is metal content of metal added.

3.5.4 Spiking teff Sample

Known amount of each metal was added from the 1000 mg/L of stock solution in to flasks containing 0.5g of teff sample to check the efficiency of digestion procedure. The procedure was as the following: 150 mg/kg of each metal (Cr, Pb, Mn , Zn, Ni, Co,Fe and Cu) which are prepared and the metals were spiked at once in to triplicate round bottomed flasks containing 0.5 g of teff sample .Then, after the spiked samples were digested simultaneously with the unspiked samples based on the digestion method for teff samples, each sample was then determined for their respective metals (Cr, Pb, Mn, Zn, Ni, Co and Cu) and Fe by FAAS and UV-VIS, respectively. The percentage recovery of metals determination was obtained as shown in the **Table 6.**

3.6 Statistical Analysis

Microsoft Excel was used for statistical analysis. Statistical evaluations such as mean, standard deviation and relative standard deviation have done for all parameters of teff samples. All analyses were carried out in triplicate. Methodological precision was therefore evaluated with standard deviation (SD). Statistical analysis of the data were carried out using one-way analysis of variance (ANOVA) to assess significant variation in the mean concentrations of selected metals in teff samples. A Probability level of $p < 0.05$ was considered statistically significant. Pearson's correlation coefficient was used to determine the association between the selected metals in teff samples.

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1 Method Validation

The efficiency of the optimized procedure and analytical method were checked by adding known concentration of each metal in 0.5 gram of teff sample. As shown in **Table 6**, the results of percentage recoveries for the studied metals in teff samples lies within the range 99.81 – 113.35 %, which is in the acceptable range (99.81 – 113 %), this confirms that, the laboratory performance for each analyte is in control and the optimized wet digestion method is valid[56]. Therefore, the optimized digestion procedure was valid for the teff samples and is believed to remove metal fractions associated with organic matter.

Table 6: Recovery Values of Metals for the Analyzed teff Samples

Metals	**Conc.in sample (mg/kg)**	**Amount added (mg/kg)**	**Conc.in spiked sample (mg/kg)**	**% Recovery**	**RSD**	**Method used**
Pb	2.16± 0.025	150	152.21 ± 0.161	100.04 ± 0.105	0.107	FAAS
Co	5.36±0.284	150	175.33±0.0057	113.32±0.0038	0.003	FAAS
Cr	4.73±0.020	150	154.21±0.1619	99.94±0.033	0.033	FAAS
Ni	6.15 ± 0.036	150	156.08 ± 0.080	99.95±0.053	0.053	FAAS
Cu	112.46 ± 0.152	150	262.53 ± 0.378	99.81 ± 0.635	0.637	FAAS
Mn	481.36 ± 0.208	150	632.34 ± 0.001	100.65± 1.15	1.147	FAAS
Zn	154.36±0.0251	150	304.35±0.0057	99.99± 0.0038	0.004	FAAS
Fe	697.26± 0.025	150	867.26 ± 1.015	113.35 ± 0.016	0.014	UV – VIS

4.2 Determination of metal concentrations in white, red and mixed teff sample

The data presented in table 7 shows that the essential and non- essential metal content had a slight difference across sampling sites. This slight difference distribution pattern of the metals in red teff sample might be accounted for factors like competition of uptake of the elements by the plant bioavailability, physical property and elemental content of the soil and soil PH [45, 55].

Table 7: Rpresents the concentration of each studied metal in the Teff samples collected from two selected sites of Amhara Regional state in West Gojam Zone, Yilmana Densa Woreda (Kililt and fetlo kuskaum). Each value represents the average of (replicates (n) and the outcomes expressed as mean values±(deviation (SD) in mg/kg in dry weight. In the study, seven essential metals (Fe, Mn, Zn, Cu, Cr, Ni, and Co) and one non-essential metal (Pb) were investigated using AAS.

Table 7: Summary of the detected mean level of each studied metal (mg/kg dry weight) in the white, Red and mixed teff samples from the two sites

Metals	Sampling site					
	Kililt			fetlo kuskaum		
	White	Mixed	Red	White	Mixed	Red
Pb	1.14 ±0.02	1.74±0.02	2.16± 0.02	1.93±0.015	2.26±0.04	1.41 ± 0.03
Ni	6.15± 0.03	8.16± 0.02	10.81± 0.02	8.38 ± 0.02	5.15 ± 0.01	4.15± 0.02
Co	5.36±0.01	5.73 ± 0.02	6.26 ± 0.01	7.15± 0.01	5.61 ± 0.02	6.81± 0.03
Cu	112± 0.15	91.46± 0.30	101.4± 0.11	81.56 ± 0.15	75.53 ± 0.25	82.43±0.41
Mn	481.3±0.2	516.8±0.28	225.5 ±0.17	316.6±0.15	191.4 ± 0.20	212.5± 0.05
Fe	697.2±0.02	723.5±0.03	651.3±0.02	681.5±0.01	632.7±0.015	761.5 ±0.01
Cr	4.73 ± 0.02	5.15 ± 0.01	4.36 ± 0.02	4.17 ± 0.015	3.93 ±0.015	4.54 ± 0.041
Zn	154.3± 0.2	168.4 ±0.26	137.9 ± 0.32	155.1 ± 0.11	142.7 ± 0.15	87.6 ± 0.40

4.2.1 Concentrations of metals in teff samples

The concentrations of metals in the three varieties of the teff samples were specified in Table 7 and figure 7, for Kililt and figure ,8 Fetlto kuskaum sampling site additionally the Mean values were determined from triplicate analysis of each sample. The mean concentrations of metals determined (mg/kg, dry weight) were in the ranges of 632-761,191-516, 87-168, 75-112, 4.15-10.81, 5.36-7.15, 3.93-5.15 and 1.14-2.26 for Fe, Mn, Zn, Cu, Ni, Co, Cr and Pb were mg/kg respectively. The concentrations of the metals showed that the red teff samples collected from fetlo kuskaum have higher amounts of Fe and Cu, compared to that of the white and mixed teff samples. The mixed teff samples collected from Kililt has higher amounts of Fe, Mn and Zn, compared to that of the red and white teff samples. Pb is another toxic metal to humans. Pb was also detected in the teff samples at a trace level (1.14 – 2.26mg/kg). The presence Pb at the trace levels in the teff samples is not desirable and hence it undermines the nutritional values of the teff to some extent.

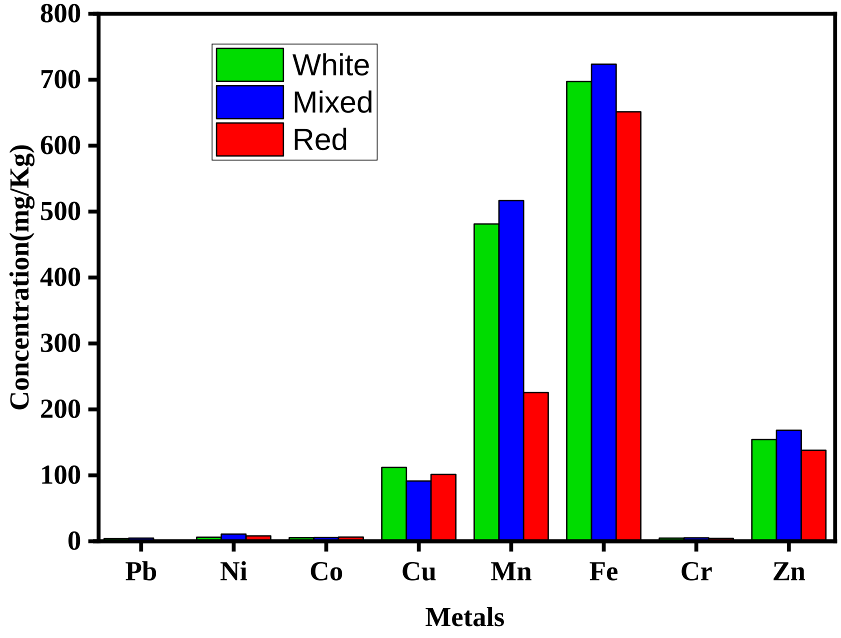


Figure 7: Spreading pattern of metals Fe, Mn, Zn, Cu, Ni, Cr, Co and Pb in white, red and mixed Teff sample in Kililt sampling site

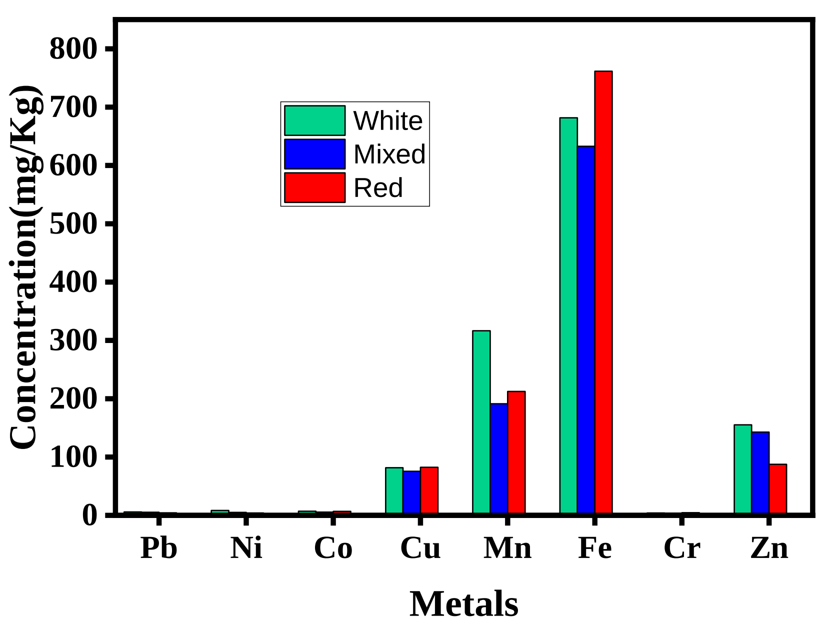


Figure 8: Spreading pattern of metals Fe, Mn, Zn, Cu, Ni, Cr, Co and Pb in white, red and mixed Teff sample in fetilo kuskaum sampling site.

COMPARISON OF LEVELS OF METALS IN TEFF WITH OTHER CEREALS

Table 8 shows that when the investigated metals were compared to other cereals such as maize, sorghum, wheat, and rice, different concentration ranges were observed. Equivalent Zn concentrations are found between teff and rice, wheat, and teff; equivalent Cu concentrations are found between teff and maize. There have been notable variations in the higher amounts of the other metals between teff and maize, sorghum, wheat, and rice. Variations in agricultural practices and species variety could be the cause of this difference. The results of this investigation showed that teff had higher levels of Fe, Cu, and Zn than grains like sorghum, wheat, and rice. Teff contains more iron than maize and sorghum, zinc equivalent to wheat, and copper comparable to maize.

Table 8: Comparison of the mineral content of teff grain compared to other cereals (mg/kg)

Cereals	Metal concentration (mg/Kg)			Methods	References
	Fe	Cu	Zn		
White teff	681.5-697.2	81.56-112	154.3-155.1	FAAS	This study
Red teff	723.5-761.5	82.43-91.46	87.6-168.4	FAAS	This study
Mixed teff	632.7-651.3	75.53-101.4	137.9-142.7	FAAS	This study
Maise	36-48	13	26-46	FAAS	10
Maise	18-115	0.04-1.32	61.7-77.6	GF	25
Sorghum	35-41	4.1	14-17	AAS	56
Wheat	37	2.3	17	FAAS	2
Rice	15	1.6	22	FAAS	10
Rice	41-113	3.3-15	17-140	FAAS	56
White teff	95-377	25-53	24-68	FAAS	2
Red teff	116-1500	11-36	23-67	FAAS	1
Mixed teff	115-1500	16	38-39	FAAS	56

COMPARISONS OF THE METAL CONTENTS IN TEFF SAMPLES WITH OTHER REPORTED VALUES

The Fe concentrations identified in the teff samples used in this investigation, as indicated in **Table 9**, are within the range of the study conducted by [13] and are lower than the other published values. The study's determination of Cr is comparatively less than the other values that have been published. The Cu amount determined by this method is almost higher than the other values that have been published. [39] also measured the Pb content of teff; their results were lower than the Pb levels found in this investigation. The Mn found in this investigation is greater than the other research's stated levels. The amount of zinc found in this investigation is higher than the results previously reported.

Table 9: Comparison of the mineral (mg/kg, dry mass) of teff with reported values

Cereals	Metals concentration (mg/Kg)			Methods	References
	Fe	Cu	Zn		
White teff	681.5-697.2	81.56-112	154.3-155.1	FAAS	This study
Red teff	723.5-761.5	82.43-91.46	87.6-168.4	FAAS	This study
Mixed teff	632.7-651.3	75.53-101.4	137.9-142.7	FAAS	This study
White	11.4	25-53	9.58	FAAS	14
Red	246 ± 1	11-36	23.67	FAAS	14
mixed	201 ± 1	16	38-39	FAAS	13
White teff	95-377	25-53	30 ± 0.12	FAAS	20
Red teff	116-1500	11-36	48 ± 11	FAAS	20
Mixed teff	115-1500	16	38 ± 0.1	FAAS	20

Table 10: Metal concentrations in teff (this study), the amount that an average adult man can get from 180 g teff per day, RDI and upper limit values of metals recommended by experts and agencies for a normal adult man.(FAO/WHO, 2011 and www.frantzmd.info, www.

Metal	Concentration in teff (mg/Kg)	Amount of metal a person can get from 180g teff (mg)	Recommended daily intake (RDI)	**Tolerable upper limit**
Pb	**1.14-2.26**	**0.32-0.38**	**0.02-3 µg/kg b.w/day**	**25 µg/kg b.w/day**
Ni	4.15-10.81	**NR**	**NR**	**NR**
Co	5.36-7.15	**NR**	**NR**	**NR**
Cu	75-112	**2.3-2.6**	**0.9-2.3 mg**	**5 mg/day**
Mn	191-516	**3.6-5.0**	**1.8-2.3 mg**	**11 mg/day**
Fe	632-761	**87-215**	**9-17 mg**	**45 mg/day**
Cr	3.93-5.15	**NR**	**NR**	**NR**
Zn	87-168	**13-15**	**10-20 mg**	**40 mg/day**

NR= Non Reported

4.2.2 Spreading pattern of essential metals Mn, Fe, Zn, Cu, Ni, Cr and Co in white, red and mixed Teff sample

As specified in **figure 7,** the concentration level of Fe was higher than those essential metals investigated throughout the two sample sites. Its concentration range was 632-761mg/kg dry weight, followed by Mn (191-516mg/kg), and Zn (87-168 mg/kg). The distribution pattern of essential metals determined in the sample were follow decreasing order of Fe >Mn >Zn>Cu>Ni>Co>Cr. Among the sample sites the highest concentration of Fe was obtained from fetlo kuskaum (761.5±0.01mg/kg) followed by Kililt (697.2±0.02mg/kg). The distribution pattern of Fe concentration in the sample by sampling site decrease in the order of fetlo kuskaum > Kililt. The concentration level of Zn analyzed in the sample was ranged from 87.6 to 168.4 mg/kg. The distribution pattern of Zn concentration in red teff sample by the sampling site decrease in the order of Kililt >felto kuskaum.

4.3 Analysis of variance (ANOVA)

The significance of variation between samples was analyzed using one-way ANOVA (Kyplot software). From ANOVA the mean concentrations of all metals (Co, Cr, Pb Ni, Fe, Mn, Cu and Zn) were showed significant difference ($P < 0.05$ at 95% confidence interval) between different types of teff (**table 11).**

This difference contest with the accumulation of metals in soil at which teff is cultivated. The presence of significant differences may be due to the presence of different geographical distribution, rainfall, soil composition, harvesting, and storing methods.

Table 11: ANOVA Table of Selected Metals in teff sample (at 0.05 levels)

Zn	Between Groups Within Groups Total	1451.087 0.473333	2 6 8	725.5433 0.078889	9197.02	5.143253	3.47E-11
Cu	**Between Groups** **Within Groups** **Total**	662 0.26	2 6 8	331 0.043333	7638.46	5.143253	6.05E-11
Mn	**Between Groups** **Within Groups** **Total**	151361.4 0.313333 151361.7	2 6 8	75680.68 0.052222	144920	5.143253	8.87E-18
Fe	**Between Groups** **Within Groups** **Total**	8005.432 0.004 8005.436	2 6 8	4002.716 0.000667	600407	5.143253	1.25E-19
Ni	**Between Groups** **Within Groups** **Total**	32.7782 0.0042 32.7824	2 6 8	16.3891 0.0007	23413	5.143253	2.1E-12
Pb	**Between Groups** **Within Groups** **Total**	1.566022 0.003 1.569022	2 6 8	0.783011 0.0005	1566.02	5.143253	6.99E-09
Cr	**Between Groups** **Within Groups** **Total**	0.929689 0.0026 0.932289	2 6 8	0.464844 0.000433	1072.71	5.143253	2.17E-08
Co	**Between Groups** **Within Groups** **Total**	1.266289 0.0022 1.268489	2 6 8	0.633144 0.000367	1726.75	5.143253	5.22E-09

4.4 Pearson correlation of metals

In this study, to correlate the effect of one metal concentration on the concentration of another metal, the Pearson correlation matrices using correlation coefficient (r) for the samples were used and presented in **Table 12.** In the teff samples analyzed for the metal levels, the correlation was strong (close to ±1) for some metals, moderate (around ± 0.5) for some other metals and weak (< ±0.5) for the remaining metals. The poor relationship may be due to the fact that different soil types, environmental conditions and capacity of the plant to accumulate specific metal. The Pearson product-moment correlation coefficient is the measure of the strength of a linear association between two variables. A correlation coefficient of +1.0 indicates a perfect positive correlation while a correlation coefficient of −1.0 indicates a perfect negative correlation. Linear regression correlations tests were performed to investigate the correlations among the overall mean metal concentrations in the three varieties of teff samples collected from the two sampling areas. The results are summarized in **Table 12.** According to this study the highest correlation was seen between Ni with Cu and Pb and also Fe with Zn and Mn among the three types of teff.

Table 12: Correlation Coefficient Strength among Different Metals Concentrations in the Study Areas

variety	Cu	Pb	Zn	Mn	Ni	Cr	Co	Fe
Cu	1							
Pb	-0.99737	1						
Zn	-0.43301	0.366575	1					
Mn	-0.08441	0.012008	0.934723	1				
Ni	-0.99432	0.984001	0.526471	0.189966	1			
Cr	0.441249	-0.37508	-0.99996	-0.93143	-0.53424	1		
Co	-0.99068	0.978213	0.551742	0.219337	0.99955	0.55936	1	
Fe	-0.33381	0.264651	0.994229	0.967453	0.432227	-0.9932	0.459088	1

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In this study the level of Mn, Fe, Zn, Pb, Cu, Ni, Cr and Co in white, mixed and red Teff samples collected from Yilmana Densa Woreda, West Gojjam Zone,Ethiopia were determined by FAAS. The digestion procedure applied for the determination of metals in teff samples were validated through spiking method and percentage recovery was obtained (99.81 – 113 %) for essential and non-essential the metals of interest. The mean concentrations of metals determined (mg/kg, dry weight) were in the ranges of Fe, Mn, Zn, Cu, Ni, Co, Cr and Pb were 632-761,191-516, 87-168 , 75-112, 4.15-10.81, 5.36-7.15, 3.93-5.15 and 1.14-2.26 mg/kg respectively. The concentrations of the metals showed that the red teff samples collected from fetilo kuskaum have higher amounts of Fe and Cu, compared to that of the white and mixed teff samples. Analysis of variance indicated that there is significant difference between the mean concentrations of all metals (Co, Cr, Pb Ni, Fe, Mn, Cu and Zn) were showed significant difference ($P < 0.05$ at 95% confidence interval) between different types of teff. The results show that teff grain is good source of essential metals with the toxic metal Pb, above WHO guidelines 0.3 mg/kg concentrations. It was higher than the FAO/WHO safe limit. Fe was the most accumulated metal in study areas, followed by Mn, but high concentration of Fe and Cu were recorded in Fetilo Kuskuam, As compared to the previous report. Levels of essential metals were found in the three types of teff samples in this study. It was also more than the concentration of metals reported in other cereals. Therefore, from this study, teff grains are a good source of essential metals with further investigation of the toxicity of Pb metal. The presence of significant differences may be due to the presence of different geographical distribution, rainfall, soil composition, harvesting, and storing methods.fertilizer,pesticids and herbicides.

5.2. Recommendation

Typical sample on white. Mixed and red teff from different geographical conditions (soil type, soil PH and climate) has not yet studied. Therefore, it is recommended that the results of this study be used as a stepping stone for further research. As teff is important to the community, agricultural research centers should work with the community to improve teff production. Further research is recommended to examine the safety and toxicity of these metals, which have concentration more than the FAO/WHO safe limit.

REFERENCE

1. Grant, G., *Bombers' Bazaar*. Foreign Policy, 2006(154): p. 93.
2. Ketema, S., *Tef-Eragrostis tef (Zucc.)*. Vol. 12. 1997: Bioversity international.
3. Abebe, A. and B.S. Chandravanshi, *Levels of essential and non-essential metals in the raw seeds and processed food (roasted seeds and bread) of maize/corn (Zea mays L.) cultivated in selected areas of Ethiopia.* Bulletin of the Chemical Society of Ethiopia, 2017. **31**(2): p. 185-199.
4. Abebe, Y., et al., *Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability.* Journal of food composition and analysis, 2007. **20**(3-4): p. 161-168.
5. Nordberg, G., *Cadmium and human health: a perspective based on recent studies in China.* The Journal of Trace Elements in Experimental Medicine: The Official Publication of the International Society for Trace Element Research in Humans, 2003. **16**(4): p. 307-319.
6. Abraham, R., *Achieving food security in Ethiopia by promoting productivity of future world food tef: A review.* Adv Plants Agric Res, 2015. **2**(2): p. 00045.
7. ADDISU, E.A., *Determination of Essential And Non-Essential Elements in Red Teff in Sire Woreda, Arsi Zone, Ethiopia*, 2021.
8. Al-Fartusie, F.S. and S.N. Mohssan, *Essential trace elements and their vital roles in human body.* Indian J Adv Chem Sci, 2017. **5**(3): p. 127-136.
9. Ali Taher, M., *Flame atomic absorption spectrometric determination of trace lead after solid-liquid extraction and preconcentration using 1-(2-pyridylazo)-2-naphthol.* Croatica chemica acta, 2003. **76**(3): p. 273-277.
10. Asfaw, Z. and A. Gatahun, *Biology and human welfare: Domestication of plants by humans.* Biology, 2000: p. 229-246.
11. Assefa, B., *Analysis of price incentives for teff in Ethiopia for the time period 2005–2012.* Gates Open Res, 2019. **3**(109): p. 109.
12. Awulachew, M.T., *Teff (Eragrostis Abyssinica) and teff based fermented cereals.* Journal of Health and Environmental Research, 2020. **6**(1): p. 1-9.
13. Baye, K., et al., *Changes in mineral absorption inhibitors consequent to fermentation of E thiopian injera: implications for predicted iron bioavailability and bioaccessibility.* International Journal of Food Science & Technology, 2014. **49**(1): p. 174-180.

14. Belmonte, K.E., *Insulin modulates function of pulmonary neuronal inhibitory M (2) muscarinic receptors*. 1998: The Johns Hopkins University.
15. Bernhoft, A., et al., *Trace elements in reindeer from Rybatsjij Ostrov, north western Russia.* Rangifer, 2002. **22**(1): p. 67-73.
16. Chaltu, G. and B. Abraham, *Yeast flora of fermenting tef (Eragrostis tef) dough.* SINET: Ethiop. J. Sci, 1982. **5**: p. 21-25.
17. Costanza, S.H., *Literature and numerical taxonomy of t'ef (Eragrostis tef),* 1978, University of Illinois at Urbana-Champaign.
18. Council, N.R., C.o.L. Sciences, and S.o.t.T.E.o.t.R.D. Allowances, *Recommended dietary allowances.* 1989.
19. Curtis, K., et al., *Market potential for Nevada teff production. Technical Raport Un.* Center for Economic Development. Un. Nevada, Reno. NV, 2008.
20. Das, K., S. Das, and S. Dhundasi, *Nickel, its adverse health effects & oxidative stress.* Indian journal of medical research, 2008. **128**(4): p. 412-425.
21. Tadele, E. and T. Hibistu, *Empirical review on the use dynamics and economics of teff in Ethiopia.* Agriculture & food security, 2021. **10**: p. 1-13.
22. Mesfin, B., *Women\'s participation in political leadership and decision-making in Ethiopia: A research note.* Ethiopian journal of the social sciences and humanities, 2004. **2**(2): p. 80-99.
23. Mengesha, M.H. and A. Guard, *Development of the embryo sac and embryo of teff, Eragrostis tef.* Canadian Journal of Botany, 1966. **44**(8): p. 1071-1075.
24. Tefera, H., G. Belay, and M. Sorrells, *Narrowing the Rift: Tef Research and Development: Proceedings of the" International Workshop on Tef Genetics and Improvement", Debre Zeit, Ethiopia, 16-19 October, 2000*. 2001: Ethiopian Agricultural Research Organization.
25. Duda-Chodak, A. and U. Blaszczyk, *The impact of nickel on human health.* Journal of Elementology, 2008. **13**(4): p. 685-693.
26. El-Alfy, T.S., S.M. Ezzat, and A.A. Sleem, *Chemical and biological study of the seeds of Eragrostis tef (Zucc.) Trotter.* Natural Product Research, 2012. **26**(7): p. 619-629.
27. Engdawork, T. *Understanding Tef: A Review of Supply and Marketing Issues. Ethiopia Commodity Exchange Authority*. in *Proceeding of the" Inernational Workshop on Teff Genetics and Improvement*. 2009.

28. Eshetu, G., *Commercialization of Smallholder Teff Producers in Jamma District, South Wollo Zone, Ethiopia*, 2018, Haramaya university.
29. Joint, F., *Human vitamin and mineral requirements.* 2002.
30. Gebremariam, M.M., M. Zarnkow, and T. Becker, *Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review.* Journal of food science and technology, 2014. **51**: p. 2881-2895.
31. Gizaw, W. and D. Assegid, *Trend of cereal crops production area and productivity, in Ethiopia.* Journal of Cereals and Oilseeds, 2021. **12**(1): p. 9-17.
32. Godt, J., et al., *The toxicity of cadmium and resulting hazards for human health.* Journal of occupational medicine and toxicology, 2006. **1**: p. 1-6.
33. Guéguen, L. and A. Pointillart, *The bioavailability of dietary calcium.* Journal of the American College of Nutrition, 2000. **19**(sup2): p. 119S-136S.
34. Hager, A.-S., et al., *Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours.* Journal of Cereal Science, 2012. **56**(2): p. 239-247.
35. Harlan, J.R., *Origins of African plant domestication*. 2011: Walter de Gruyter.
36. Hopman, E., et al., *Tef in the diet of celiac patients in The Netherlands.* Scandinavian journal of gastroenterology, 2008. **43**(3): p. 277-282.
37. Dagnaw, H.B., A.Z. Woldegiorgis, and K.A. Kebede, *Influence of nitrogen fertilizer rate and variety on tef [Eragrostis tef (Zucc.) Trotter] nutritional composition and sensory quality of a staple bread (Injera).* Plos one, 2024. **19**(1): p. e0295491.
38. Cholakova, A., T. Zhelyazkova, and M. Gerdzhikova, *Significance of teff (Eragrostis tef (Zucc.) Trotter) as a new fodder crop for Bulgaria.* Agricultural Science & Technology (1313-8820), 2023. **15**(4).
39. Kibatu, G., R. Chacha, and R. Kiende, *Determination of major, minor and trace elements in Tef using portable total x-ray fluorescence (TXRF) spectrometer.* EC Nutrition, 2017. **9**(1): p. 51-59.
40. Lovis, L., *Alternatives to wheat flour in baked goods.* Cereal Foods World, 2003. **48**(2): p. 61.
41. Mason, R., *The Minerals You Need.* USA: Safe Goods Publishing, 2011. **2**: p. 45-78.
42. Meacham, S.L., L.J. Johnson, and L.J. Kruskall, *Recommended dietary allowances-then and now: a review.* Journal of Agromedicine, 2002. **8**(2): p. 85-93.

43. Medeiros, R.J., et al., *Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil.* Food control, 2012. **23**(2): p. 535-541.

44. Mengesha, M.H., *Chemical composition of teff (Eragrostis tef) compared with that of wheat, barley and grain sorghum.* Economic Botany, 1966. **20**(3): p. 268-273.

45. Mengistu, D.K. and L.S. Mekonnen, *Integrated agronomic crop managements to improve tef productivity under terminal drought.* Water Stress. Vienna Tech, 2011. **1**: p. 235-254.

46. Haile, M., et al. *Market access versus productivity: The case of Teff*. in *Ethiopian Economic Association, Conference on Ethiopian Economy, Addis Ababa*. 2004.

47. Molineaux, L. and M. Biru, *Tef consumption, hookworm infestation, and hemoglobin levels: a preliminary report.* J Health, 1965. **51**(1): p. 1-5.

48. Morgano, M.A., et al., *Assessment of trace elements in fishes of Japanese foods marketed in São Paulo (Brazil).* Food Control, 2011. **22**(5): p. 778-785.

49. Portères, R., *Les appellations des céréales en Afrique.* Journal d'agriculture traditionnelle et de botanique appliquée, 1958. **5**(11): p. 732-761.

50. Saturni, L., G. Ferretti, and T. Bacchetti, *The gluten-free diet: safety and nutritional quality.* Nutrients, 2010. **2**(1): p. 16-34.

51. Seyfu, K., *„Tef. Eragostis tef (Zucc.) Trotter. Promoting the conservation and use of underutilized crops". Rome: Institute Plant.* Genetics and Crop Research, 1997. **12**: p. 35.

52. Sharma, K. and E.S. Chauhan, *Nutritional composition, physical characteristics and health benefits of teff grain for human consumption: A review.* The Pharma Innovation Journal, 2018. **7**(10): p. 3-7.

53. Sneddon, J., et al., *Sample preparation of solid samples for metal determination by atomic spectroscopy—an overview and selected recent applications.* Applied Spectroscopy Reviews, 2006. **41**(1): p. 1-14.

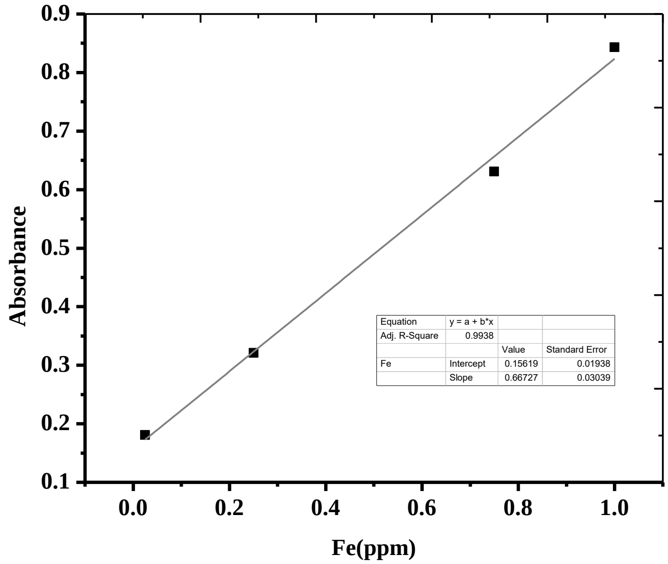
54. Spaenij-Dekking, L., Y. Kooy-Winkelaar, and F. Koning, *The Ethiopian cereal tef in celiac disease.* New England Journal of Medicine, 2005. **353**(16): p. 1748-1749.

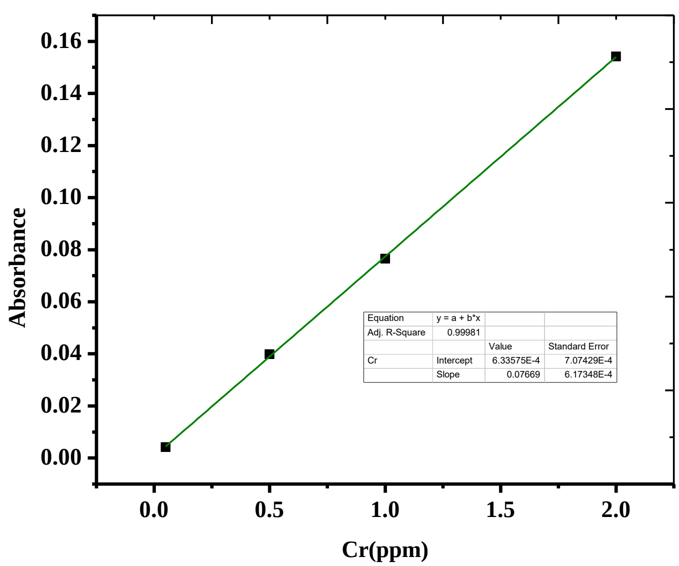
55. Tefera, H., *Inheritance of morphological and agronomic traits in tef (Eragrostis tef).* 2002.

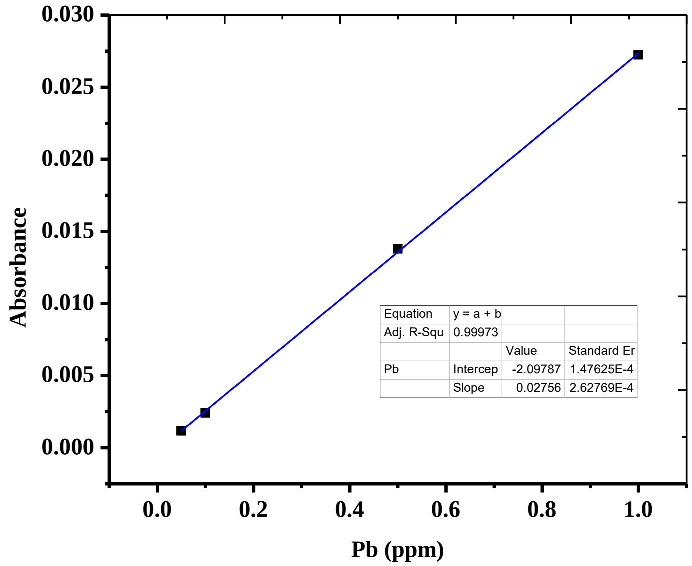
56. Tegene, B.G., et al., *Farmers traditional knowledge on teff (Eragrostistef) farming practice and crop rotation in PGP microbes enhancement for soil fertility in West and East Gojam.* Computational Biology and Bioinformatics, 2016. **4**(6): p. 45-54.

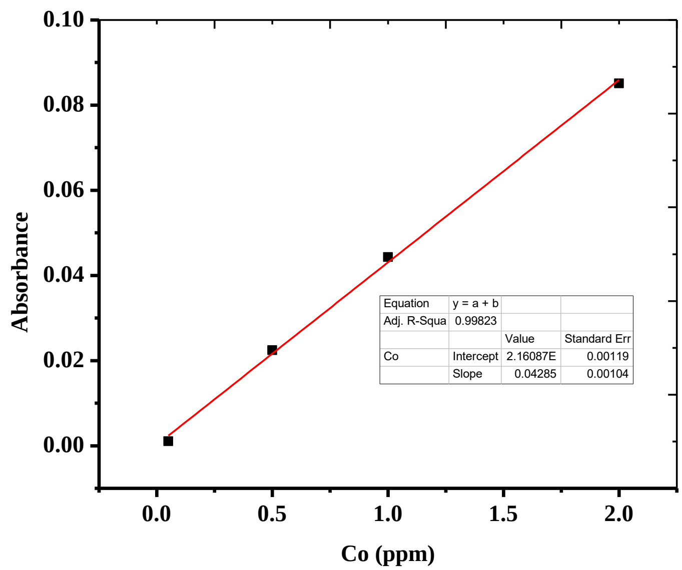
Appendix I

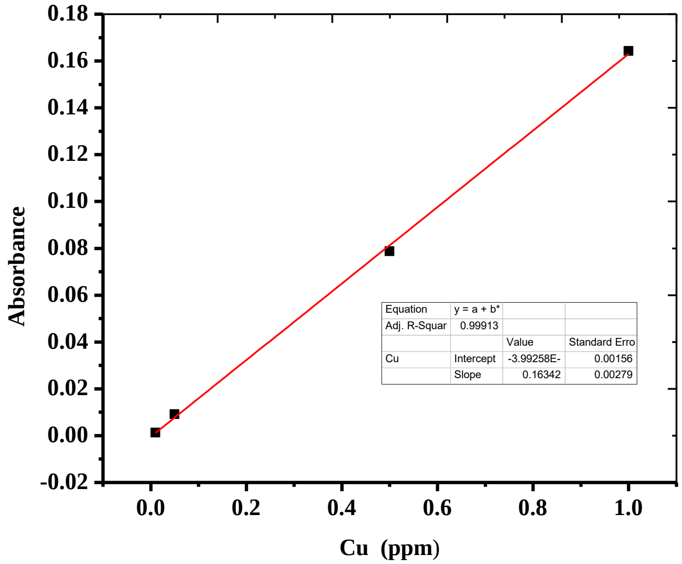
Calibration curves of working standard concentration of metals versus absorbance



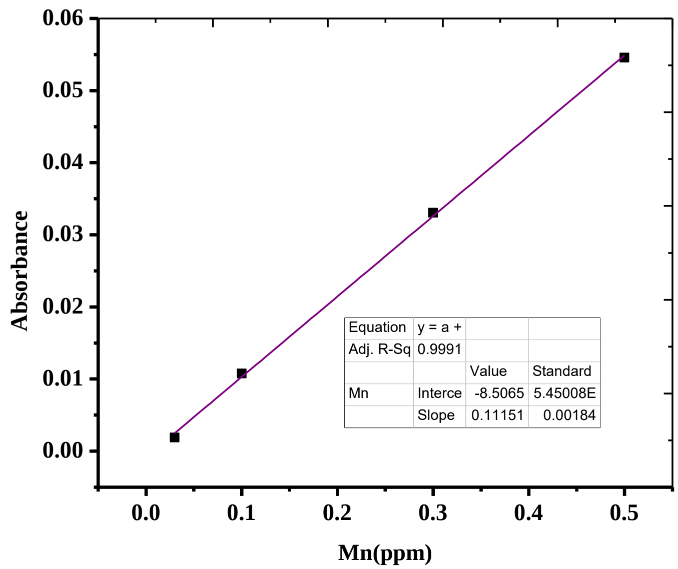



0.030
0.025
0.020
0.015
0.010
0.005
0.000
Absorbance
0.0
0.2
0.4
0.6
0.8
1.0
Pb (ppm)
Equation | y = a + b
Adj. R-Squ | 0.99973
Value | Standard Er
Pb | Intercep | -2.09787 | 1.47625E-4
Slope | 0.02756 | 2.62769E-4

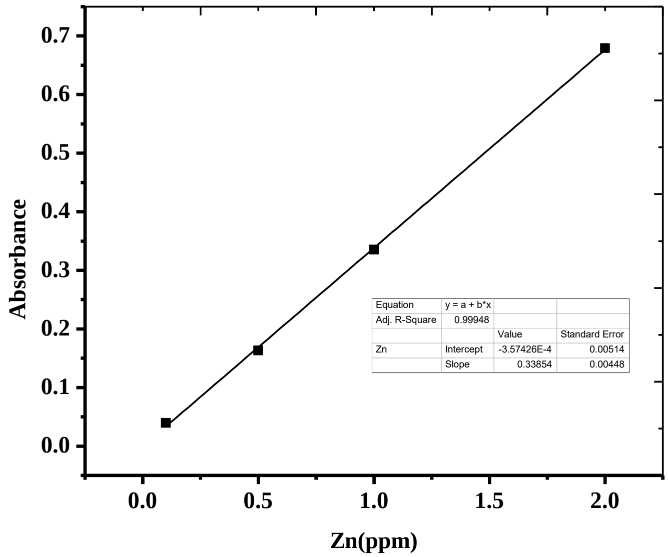

0.10
0.08
0.06
0.04
0.02
0.00
Absorbance
0.0
0.5
1.0
1.5
2.0
Co (ppm)
Equation | y = a + b
Adj. R-Squa | 0.99823
Value | Standard Err
Co | Intercept | 2.16087E | 0.00119
Slope | 0.04285 | 0.00104



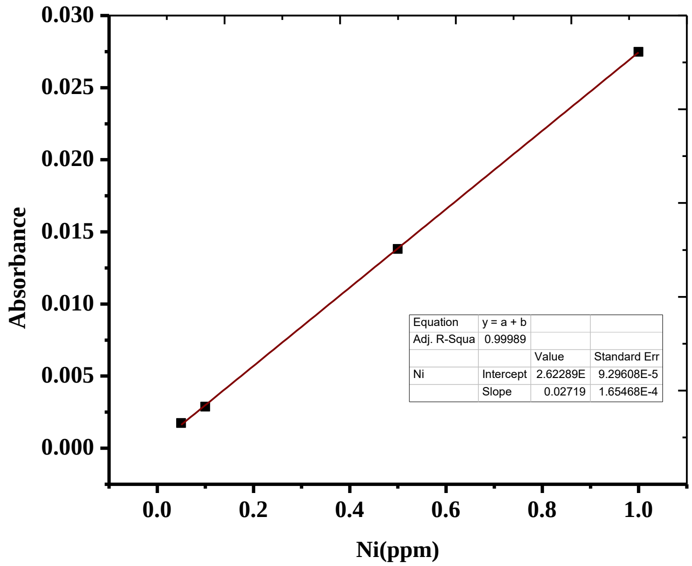
Absorbance
Cu (ppm)
Equation y = a + b*
Adj. R-Squar 0.99913
Value Standard Erro
Cu Intercept -3.99258E- 0.00156
Slope 0.16342 0.00279



Absorbance
Mn(ppm)
Equation y = a +
Adj. R-Sq 0.9991
Value Standard
Mn Interce -8.5065 5.45008E
Slope 0.11151 0.00184



0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbance
0.0
0.5
1.0
1.5
2.0
Zn(ppm)
Equation
y = a + b*x
Adj. R-Square
0.99948
Value
Standard Error
Zn
Intercept
-3.57426E-4
0.00514
Slope
0.33854
0.00448



0.030
0.025
0.020
0.015
0.010
0.005
0.000
Absorbance
0.0
0.2
0.4
0.6
0.8
1.0
Ni(ppm)
Equation
y = a + b
Adj. R-Squa
0.99989
Value
Standard Err
Ni
Intercept
2.62289E
9.29608E-5
Slope
0.02719
1.65468E-4