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DETERMINATION OF ESSENTIAL AND NON-ESSENTIAL METALS IN RED TEFF GROWN IN ENEMAY WEREDA, EAST GOJJAM ZONE, AMHARA REGION OF ETHIOPIA

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BY: Meseret Alemu

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A THESIS SUBMITTED TO THE DEPARTEMENT OF CHEMISTRY PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

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BAHIR DAR UNIVERSITY
COLLEGE OF SCIENCE
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October, 2021
Bahir Dar, Ethiopia

DECLARATION

I declare that this thesis entitled "Determination of Essential and Non-Essential metals in red teff grown in Enemay wereda, east Gojjam zone, Amhara Region of Ethiopia" is my own original work done under the supervision of Dr. Tihitinna Asmellash at the Chemistry Department of Bahir Dar University and I have not previously submitted it entirely or in part for obtaining any qualification at any other university.

Name: Meseret Alemu Date________________ Signature_______________

LETTER OF APPROVAL

The thesis entitled Determination of Essential and Non-Essential metals in red teff grown in Enemay wereda, east Gojjam zone, Amhara Region of Ethiopia presented by Meseret Alemu has been accepted as the partial fulfillment for the degree of Master of Science in Chemistry.

BOARD OF EXAMINERS

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LIST OF ABBRIVATION AND ACRONYMS

ANOVA	Analysis of Variance
DF	Degree of freedom
DRI	Dietary Reference Intake
FAW	Food and agriculture organization
HSD	Honestly Significant Difference
ICP – OES	Inductively coupled plasma optical emission spectroscopy
MDL	Method detection limit
LOQ	Limit of quantification
R	Correlation Coefficient
SD	Standard deviation
WHO	World health organization

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ABSTRACT

Teff, (Eragrostis tef), Ethiopia's earliest indigenous staple food, is one of essential crops for food, farm income and nutrition security in Ethiopia. Teff is very nutritious and is an important part of Ethiopia's cultural heritage and national identity. In this study, the concentration of selected essential (Mg, Cr, Mn, Fe, Ni, Cu and Zn) and non-essential (Cd and Pb) metals were determined in red teff samples collected from in Enemay Wereda, East Gojjam Zone, Amahara Region using ICP-OES. The results were compared with the metal concentrations of teff verities and other cereals. after proper sample pretretment 0.5 g of oven-dried red teff sample was digested with an optimized wet-digestion procedure using 3 mL of HNO_3 *(69-70%) and 2 mL* H_2O_2 *(30%) at 120 °C for 2:30 h.. The method was validated by its linear range in the concentration range of 0.05-5.05 ppm and the optimized procedure was evaluated by spiking experiment; the percentage recoveries ranged from 80 to 120%, indicating good accuracy. The concentrations of metals (mg/kg) in red teff samples from the five study farmlands ranged Mg (1,599 - 1,830 mg/kg), Fe (167 - 2,341 mg/kg), Mn (49.4 - 139mg/kg), Zn (27.5 - 73.5 mg/kg), Cr (1.2 - 41.9 mg/kg), Ni (8.6 - 24.3 mg/kg) and Cu (6.0 - 21.3 mg/kg). Among the metals identified Mg was found to be the highest determined metal in the samples of all five sites. Hence, Mg is the most abundant element followed by Fe and Mn. However, the non-essential metal Pb and Cd were not detection at all. One-way ANOVA revealed significant differences* ($p < 0.05$) *among the mean concentrations of metals in all red teff samples.*

Key words: *Eragrostis tef, essential metal, non essential, ICP-OES, Wet digestion*

1. INTRODUCTION

1.1. Background of the study

Teff, (Eragrostis tef), Ethiopia's earliest indigenous staple meals, it is far critical plants for meals, farm earnings and nutrients security. Teff may be very nutritious and is a critical a part of Ethiopia's cultural history and country wide identity. Being categorized as one of the most up-to-date wonderful meals of the twenty first century, teff's worldwide recognition is hastily rising (Collyns, 2013). Teff is likewise being full-grown in India, South Africa, Canada and Australia for each animal feed and human consumption. Moreover, teff is desired for its nutritional profile and is rapid recognition in Western international locations (Stallknecht, 1997; Roseberg *et al*., 2005).

In Ethiopia, teff is mostly cultivated in Oromia and Amhara with smaller quantities in the Tigray and southern kingdom and nationality people (SNNP) regions. The Central and South Tigray zones are the principal teff producing zones in Tigray (CSA, 2012). While, inside the Amhara Region, East Gojjam, West Gojjam, South Gonder, North Gonder, South Wollo, North Wollo, North Showa and Awi Zones are the principal cultivators of teff. In Oromia area the main teff producing zones include the East Shoa, West Shoa, South West Shoa, North Shoa, East Wallega, Horo Guduroo Wallega, Jimma, Illubabor and Arsi (CSA, 2012).

Climatically teff is an annual, warm-season, cereal crop adapted to a wide range of environmental conditions – tolerant of drought-stressed, water-logged and low-fertility soils. Under native habitat and for maximum production, teff performs best with a day length of 12 hours and a temperature of 10 – 27 °C. The recommended seeding rate is 15 kg ha^{-1} at a planting depth of 0.6 – 1.3 cm. Teff germinates hastily, rising inside three – 7 days. When the vegetative a part of the plant turns yellow, it's far harvested through hand (Ketema, 1997).

Teff used to make staple injera, which affords about two-thirds of the weight loss program in Ethiopia (Gamboa and van-Ekris, 2008). In addition to those different conventional makes use of of teff flour is for the arrangements porridge and nearby alcoholic drinks referred to as tela and katikala. Teff straw is used as animal feed, to plaster dust huts and to make nearby grain garage silos referred to as goiters (Ketema, 1997). Moreover, teff is a super supply of critical amino acids, and it includes better lysine content material, an amino acid that is maximum often deficient in cereals, than different grains (Jansen *et al*., 1962). And also, it has a better content material of iron, calcium, phosphorus, copper, and zinc as compared to different grains like wheat, barley, and sorghum (Mohammed *et al*., 2009). It is likewise suggested to be free from gluten (Mengesha, 1996; Spaenij-Dekking *et al*., 2005) and can be appropriate to be used with inside the weight loss program of sufferers struggling with celiac disease (Mamo and Parsons, 1987; Hopman et al., 2008). Teff proteins are non-gluten in nature. It has excessive dietary content material along with all critical amino acid composition especially lysine, greater mineral content material (mainly iron, calcium, phosphorus and copper) than different cereal grains. It includes B1 diet and is wealthy in fiber (Gamboa *et al*., 2008).

In Ethiopia, There are numerous styles of teff, amongst those the 3 principal classes may be recognized are white (nech), red (quey) and mixed (sergegna). Consumers the use of those sorts for specific functions in diverse form along with injera, porridge, and bread. Mostly, red teff is the only much less ate up as bread (Injera) due to much less soft, porous or sponge and conventional bias which fails to understand the particular excellent characteristics. Some others ate up it believing that it is able to restore broken (fractured) bone because of injury; others are ate up as proper dietary meals for kids and pregnant mothers. In maximum of growing international locations like Ethiopia, such developments are not primarily based totally on medical evidences however on unusual practices. However, the presence of mineral nutrients in the diet gives complete nutritional aspect. Thus, a whole profile of mineral composition in red teff should be to be had for the customers accordingly. Therefore, the purpose of this study is to determine essential and non-essential metals in red teff samples grow in Enemay Wereda, East Gojjam Zone, Amahara Region of Ethiopia using inductively coupled plasma optical Emission Spectroscopy (ICP-OES).

1.2. Statement of the problem

Since teff is a staple food for many people in Ethiopia, knowledge of its mineral level is particular interest. Some studies on mineral nutrients of teff and other cereals crops have been reported by Ketema (1997) and Mengesha (1966). There have been also many studies on minerals determination in different teff varieties , but, there was little or no research conducted on assessing the mineral contents of red teff grown in East Gojjam Zone particular Enemay Wereda.

Therefore, this study is intended to determine the contents of essential and non-essential metals in red teff samples cultivated in Eest Gojam zone, Enemay woreda by using inductively coupled plasma optical Emission Spectroscopy (ICP-OES).

1.3. Objectives of the Study

1.3.1. General objective

The general objective of this study is to assess the concentration of selected essential and non-essential metals in red teff grown in five kebeles (Mankorkoya, Bechenadebir, Yekebehana, Yerese and Debissa) of Enemay wereda, Amhara region.

1.3.2. Specific objectives

The specific objectives of this study are to:

- ✓ Determine the concentration of Mg, Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb in red teff samples.
- ✓ Compare the levels of selected essential (Mg, Cr, Mn, Fe, Ni, Cu and Zn) and non-essential (Cd and Pb) metals between the five sampling sites.
- ✓ Compare the levels of the studied essential and non-essential metals in the red teff sample with other teff verities from literatures.

1.4. Significance of the Study

- ✓ The results of this study will be important in formulation of mineral nutrient supplements from red teff.
- ✓ Provide the levels of essential and non-essential metals contained and the consumer will have information while using the red teff.
- ✓ The information obtained will be used to sensitize people on the role of levels of selected essential elements in red teff.

2. REVIEW LITERATURE

2.1. History and origin of teff

Teff (*Eragrostis tef*) the phrase Eragrostis is beloing to a member of the tribe *Eragrosteae*, sub-own circle of relatives *Eragrostoidae*, of the *poaceae* (*Gramineae*). There are about three hundred species with inside the genus *Eragraostis* which includes each perennials and annuals which might be located over a huge geographic variety (Jones *et al*., 978; Costanza *et al*., 1979; Bekele and Lester, 1981). The middle of starting place and variety of historic tropical cereal teff is with inside the northern Ethiopian highlands from in which its miles believed to have been domesticated (Ketema, 1997; Demissie, 2001).

In Ethiopia teff is a usually cultivated cereal crop with excessive marketplace fee and socio-monetary values. Moreover, its middle of starting place and variety is Ethiopia. Zewudu, 2008 stated that they're additionally produced in different nations which includes USA, Canada, Australia, South Africa, and Kenya for one of a kind functions like forage crop and a thickener for soups, stews, and gravies. With populace past 60 million people, Ethiopia is one of the country within the global in which teff is specially grown and produced for human feeding. Teff is a major meals in Ethiopia, which includes two-thirds in their cereal meals, and is specially used to mark injera (Ketema, 1997).

2.2. Types and Characteristics of Teff Grain

Teff is perhaps the smallest cereal crop with a median length of ~ 1 mm (Umeta and Parker, 1996; Lacey and Llewellyn, 2005; Bultosa, 2007; Adebowale *et al*., 2011). The common thousand kernel weight of 12 teff types examined with the aid of using Bultosa, 2007 become 0.264 g. The tiny teff grains has technological and dietary implications. For instance as teff grains are tough to decorticate, the cereal is ate up as a wholegrain, offers nutrient intake for purchasers. The color of teff may also range from white to darkish brown depending on its variety. In Ethiopia, 3 fundamental groups may be recognized: red (quey), white (nech) and mixed (sergegna). It is not unusual for wholesalers to subdivide white teff into very white (magna) and white (nech). White teff generally cultivated most effective inside the Ethiopian highlands and wishes rather right developing situations. Due to its better consumer preference, any individual may also rationalize white teff is the maximum high priced form of teff. However, in latest years, red teff, which is thought to be greater nutritious, is likewise gaining recognition amongst fitness aware purchasers in Ethiopia (Gebremariam, *et al*., 2012).

2.3. Production and harvesting of teff

According to BARD (beuro of agricultural and rural development), Row planting technology become introduced in 2010/2011 at farm degree. It has been argued lately that low teff productiveness is partially due to the manner farmers sow teff seed. Traditionally, farmers broadcast the seed the usage of a fee of 25 – 50 kg according to hectare MOA/ATA, 2011). This practice reduces yields because of the uneven distribution of the seeds, better competition among plants for inputs (water, light and nutrients), and difficult weeding once the plants have matured. As a solution, it's been proposed to lessen seed quotes and to plant seed in rows or to transplant seedlings (as is frequently executed for rice, for instance). Reducing the seed fee to among 2.5 and 3 kg according to hectare allows for decreased competition among seedlings and optimal tilling of the teff plants. By row planting or transplanting the seeds, land control and mainly weeding also can be executed greater quite simply and the prevalence of accommodations is decreased (MOA/ATA, 2011).

Teff grain is harvested while the vegetative a part of the plant turns yellow which usually varies among 60 and 120 days from planting. It is vital to reap earlier than the plant turns too dry to save yield losses from shattering (Ketema, 1997). It is frequently misplaced within the harvesting and threshing manner due to its length (Tadasse, 1975). In Ethiopia, the dried and harvested plant is laid out on tough, flat, cemented floor and oxen are used to thresh the crop. Oxen are pushed to and fro at the crop within the manner of isolating the grain from the head (Ketema, 1997). The grain is then separated from the straw with the aid of using tossing the grain and threshed material into the air the usage of the one of a kind aerodynamic properties, a manner recognized as winnowing. The grain is manually wiped clean with the aid of using wafting air over the grain to blow the chaff from the mixture by the usage of a tough leather-based strap (Zewdu, 2007).

2.4. Environmental Conditions for cultivation of teff grain

Teff is tailored to environments starting from drought pressure to water logged soil conditions. Maximum teff manufacturing happens at altitudes of 1800 – 2100 m, developing season rainfall of 450 – 550 mm, with a temperature variety of 10 – 27°C (Ebba, 1975; Ketema, 1997). The day period of teff is touchy and vegetation nice in the course of 12 hours of daylight (Stallknecht, 1997; Roseberg *et. al*., 2005). Teff can develop at altitudes in which many different plants can't and may be grown from sea degree to as excessive as 3000 meters altitude, with most manufacturing happening at approximately 1800 – 2100 meters (Ketema, 1997; Stallknecht, 1997). In the U.S. its grain yields common from 700 kg/ha dry land to 1400 kg/ha irrigated in Montana (Eckhoff *et al*., 1993; Stallknecht et al., 1993). Forage yields range from 9.0 to 13.5 Mg/ha, dependent upon moisture levels in the course of the developing season (Boe *et al*., 1986; Eckhoff *et al*., 1993). Teff can produce a crop in a relative quick developing season and could produce each grain for human meals and fodder for cattle (Stallknecht, 1993).

2.5. Nutritional Content of Teff Grain

Teff is a small grain which has similar meals cost with the important grains like wheat, barley and maize. Tested samples of teff have proven protein content among 14% and 15%, iron content among 11 and 33 mg and calcium content among 100 and 150 mg which might be better than maximum grains (ECHO, 2006). Another look at has additionally proven that, teff grain is tiny with an excellent supply of protein, amino acids and fiber and while in comparison to wheat, 150 grains of teff is same to at least one kernel of wheat. Nutrient wise, 2-ounce serving of teff has 7 grams of protein and the same to an extra-massive egg. Teff is better in calcium and iron content than wheat, rice, oats or millet (Hansen, 2012).

2.5.1. Carbohydrates

Based at the molecular level and degree of polymerization, carbohydrates may be categorized into sugars, oligosaccharides, starch (amylose, amylopectin), and non-starch polysaccharides. Complex carbohydrates make up 80 % of the teff grain. Carbohydrates are the important supply of strength for human nutrients and play a vital function in metabolism and homeostasis. It has a starch content of about 73%, making teff a starchy cereal. The amylose content of 13 teff types examined ranged from 20 to 26 %, akin to different grains, which includes sorghum (Bultosa, 2007).

The quantity to which carbohydrate is digested and absorbed inside the small gut determines its fitness effect. Rapidly digested and absorbed carbohydrates (glycemic carbohydrates) have more effect on blood glucose levels, as they result in more metabolic perturbation (Lafiandra *et al*., 2014). Such perturbations had been related to metabolic sicknesses which includes type-2 diabetes and cardiovascular sicknesses (Ludwig, 2002). Hence, from a fitness standpoint, slowly digesting carbohydrates are desired over swiftly digesting ones. The fee of carbohydrate digestion of a meals may be characterized with the aid of using its glycemic index (GI) (Harris and Geor, 2009).

The length of teff starch become pointed to be 2 - 6 µm (Bultosa *et al*., 2002; Wolter *et al*., 2013). This makes teff starch granules smaller than the ones of wheat (A Type 20 - 35 µm), sorghum (20 µm) and maize (20 µm) (Delcour et al., 2010). Given their large floor area, smaller starch granules are greater vulnerable to enzymatic assault (Tester *et al*., 2004). Nonetheless, in evaluation to wheat which has large starch granules, the in vitro starch digestibility of teff become located to be drastically decrease (Wolter *et al*., 2013). In line with this, the expected glycemic index of teff become drastically decrease than that of white wheat however akin to that of sorghum and oats (Wolter *et al*., 2013).

This quite decrease GI for teff than anticipated can be defined with the aid of using its amylose content, decrease starch damage, and the viable formation of amylose-lipid complexes that could prevent enzymatic get admission to and as a result starch digestibility (Singh *et al*., 2010; Wolter *et al*., 2013). In addition, the excessive (68 - 80 °C) gelatinization temperature of teff (Bultosa, 2007; Wolter *et al*., 2013) can prevent gelatinization and as a result lower susceptibility to enzymatic assault with the aid of using α-amylase (Fardet *et al*., 2006).

2.5.2. Protein

The common crude protein content of teff is within the range of 88 % to 11 %, much like different cereals which includes wheat. Teff's fractional protein composition indicates that glutelins (45 %) and albumins (37 %) are the important protein storages, at the same time as protamine are a minor constituent (~ 12 %) (Bekele *et al*., 1995; Tatham *et al*., 1996). In comparison, greater latest research record that protamine's are the important protein storages in teff (Adebowale *et al*., 2011). The one of a kind strategies of extraction among those research may also give an explanation for the contradictory findings. By analyzing the amino acid profile, the better contents of glutamine, alanine, leucine and proline and the rather decrease content of lysine in addition indicates that protamine are the important garage proteins (Adebowale *et al*., 2011).

Teff's amino acid composition is well-balanced. A rather excessive awareness of lysine, a prime proscribing amino acid in cereals, is located in teff. Similarly, in comparison to different cereals, better contents of isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine are located in teff. Another vital characteristic of teff is that it has no gluten (Hopman et al., 2008). Spaenij-Dekking *et al*., (2005) investigated the presence or absence of gluten in pepsin and trypsin digests of 14 teff types. The digests had been analyzed for the presence of T-cell stimulatory epitopes. In comparison to recognised gluten containing cereals, no T -cell stimulatory epitopes had been detected inside the protein digests of all of the teff types assayed, as a result confirming the absence of gluten in teff. This makes teff a precious factor for practical meals destined for celiac sufferers who are gluten intolerant.

2.5.3. Fat

Cereals are not the nice supply of fats, however as they're frequently ate up in massive quantities, cereals can make contributions an enormous quantity of critical fatty acids to the diet (Michaelsen *et al*., 2011). Fatty acids are doubtlessly useful to growth, improvement and long-time period fitness. Consequently, there was enormous hobby in latest years of their inclusion in diets. For instance, extended consumption of n-3 fatty acids (α-linoleic acid) had been located to lessen organic markers related to cardiovascular disease, cancer, inflammatory and autoimmune sicknesses amongst others (Simopoulos, 2001).

Rice, wheat and maize comprise negligible quantity of linoleic acid (LA) and most effective lines of α-linoleic acid (ALA). Furthermore, those extensive cereals are ate up after decortication and in addition refining which reduces their quantity of crude fats and n-6 and n-3 poly-unsaturated fatty acids. By keeping entire grains, as in the case of teff, this gives a higher supply of fatty acids than delicate ones. Teff grains are wealthy in unsaturated fatty acids, predominantly oleic acid (32.4 %) and linoleic acids (23.8 %) (El-Alfy *et al*., 2012).

2.5.4. Fiber

The American Association of Cereal Chemists defines nutritional fiber as the "fit for human consumption components of plant or analogous carbohydrates proof against digestion and absorption within the human small gut with whole or partial fermentation inside the huge gut" (DeVries, 2003). The maximum current Codex definition in addition introduced that nutritional fibers ought to have "tested physiologic results of advantage to health" (Cummings *et al*., 2009). Some of those physiologic results encompass faucal bulking (laxation), reducing blood glucose tiers after eating, and reducing plasma LDL-cholesterol (Champ *et al*., 2003).

The crude fiber, overall and soluble nutritional fiber content of teff is numerous folds better than that discovered in wheat, sorghum, rice, and maize. There can be numerous motives for this. First, complete grains have better fiber content material than decorticated ones. Second, small grains have a especially excessive percentage of bran, that's excessive in fiber (Bultosa, 2007).

2.6. Human Consumption of Teff in Ethiopia

Teff is the maximum pricey cereal in Ethiopia, justifying its prominence in city and semi-city regions wherein earning are especially better (Berhane *et al*., 2011). In Oromia region, for instance, nearby teff cultivation is the second one maximum subsequent to Amhara region. However, in Afar region, little regarded for its teff cultivation, has relatively better teff intake expenditure (10 %) (Berhane *et al*., 2011). Teff is the desired grain for making injera, usually for its higher sensory attributes (for example, taste, color, smell) and shelf life (Zegeye, 1997; Yetneberk *et al*., 2004).

Injera is made via way of means of blending cereal flour with water to make dough after which triggering the fermentation method via way of means of inoculating with ersho, a starter received from preceding fermentations. The fermentation lasts on common 2 - 3 days, and then the dough is thinned right into a batter earlier than steam baking. Besides, the capacity to without difficulty roll (softness) injera is an vital great characteristic due to the fact this permits smooth wrapping of the sauces (wot) fed on with it (Baye *et al*., 2013). In this regard, the prevalence of teff became established via way of means of the minimum pressure required to bend fresh, 24 and 48 hour saved injera relative to injera crafted from different grain (Yetneberk *et al*., 2004). Similarly, incorporating teff flour into the sorghum flour has been proven to enhance the sensory attributes of sorghum injera (Yetneberk *et al*., 2005).

Moreover, mixing teff with wheat, as is regularly located in much less privileged households (Piccinin 2002), has been discovered to be nutritionally beneficial, because it permits better phytate degradation because of the better endogenous phytase pastime in wheat (Egli *et al*., 2004). Although used to a miles lesser volume than for injera, teff also can be used for the making of porridges, unleavened breads (kitta), gruels (atmit), and conventional alcoholic beverages, like tella and arake (Gebremariam *et al*., 2012).

2.7. Minerals

Minerals are found in ingredients at low however variable concentrations and in more than one chemical forms. The function of minerals in meals is to offer a dependable supply of important vitamins in a balanced and bio-to be had form. In instances wherein attention and/or bio-availabilities in meals deliver are low, fortification has been popular (Miller, 1996). There is a widespread frame of proof that minerals via way of means of themselves and in right stability to each other have vital biochemical and dietary functions. In teff seed the distribution of mineral factors is better in the consistent with carp than in the endosperm (Mulugeta, 1979).

2.7.1. Iron:

Iron is important for red blood cells formation and required for oxygen shipping all through the frame. According to Besrat *et.al*, (1980) the iron content of 35 samples of acid washed white and red teff (additionally referred to as brown teff) grain became 3.6 to 7.8 mg/100 g on dry matter bases (DM). The distinction in mineral content among and inside teff types is extensive ranging. Red teff has a better iron and calcium content than combined or white teff (Abebe *et al*., 2007). Teff eaters have better tiers of hemoglobin of their blood, they do now no longer be afflicted by hookworm anaemia even if infested. Lovis (2003) pronounced that, teff grain carries (5.8 mg Iron/100 g) whilst wheat flour carries (4.41 mg Iron/100 g). Melak (1966) pronounced values (19.6 and 11.5mg Iron/100g) for 2 teff cultivars, whilst values (4.0 mg Iron/100 g) and (7.85 mg

Iron/100 g) for 2 wheat cultivars. According to FAO (1986) values of Iron content of teff seed ranged among (21 and 76 mg/100 g), sorghum (5 to 16 mg/100 g) and millet (39 mg/100 g).

2.7.2. **Phosphorus**:

Phosphorus works with calcium to increase and hold sturdy bones and teeth. It complements using different vitamins and play a key function in cell membrane integrity and intercellular communication. Phosphorus is essential for correct electricity approaches within the frame. Melak (1966) pronounced values (440 and 460 mg P/100 g) for 2 teff cultivars, whilst (510 mg P/100 g and 400 mg P/100 g) for two wheat cultivars. Lovis (2003) confirmed that Phosphorus content of teff grain became 378 mg/100 g whilst wheat flour (97 mg P/ 100 g).

2.7.3. Calcium and magnesium

Calcium is important for growing and preserving wholesome bones and teeth, assists in blood clotting, muscle contraction, nerve transmission and oxygen shipping. Optimal intakes lessen the threat of osteoporosis Magnesium turns on over one hundred enzymes and enables nerves and muscle functions. Lovis (2003) pronounced that teff grain carries 159 mg Ca/100 g and a 170 mg/100 g for Magnesium. While, wheat flour carries (15 mg Ca/100 g) and (25 mg Mg/100 g). Melak (1966) pronounced values of (180 mg Mg /100 g) for two teff cultivars.

2.7.4. Zinc, copper and manganese

Zinc is an important one for greater than two hundred enzymes protected within the digestion, metabolism, and duplicate and wound healing. It performs essential function in immune reaction and is a vital antioxidant. Melak (1966) pronounced that for two teff cultivars contain (6.7 and 6.8 mg Zn/100 g) while, wheat (spring and winter) carries (6 – 3.95 mg Zn/100 g). Lovis (2003) pronounced that teff grain carries (2 mg Zn/100 g). However, wheat flour carries (0.85 mg Zn/100 g).

Copper is important to ordinary red blood cells formation and connective tissue formation. It acts as a catalyst to keep and launch iron to assist hemoglobin formation. Lovis (2003) pronounced that teff grain carries (0.7 mg Cu/100 g). while, wheat flour carries (0.182 mg Cu/100 g) Melak

(1966) pronounced values (52 mg Cu /100 g and 64mg Copper/100 g) for two teff cultivars whilst (55 mg Cu /100 g) for two wheat cultivars.

Manganese is a key element of enzymes systems, guide mind characteristic and is needed for blood sugar regulation. Melak (1966) pronounced values (2.12 mg Mn/100 g) and (3.00 mg Mn /100 g) for two teff cultivars whilst (1.2 mg Mn /100 g and 3.6 mg Mn /100 g) for two wheat cultivars. Lovis (2003) pronounced that Manganese content of teff grain became (6.4 mg/100 g) and for wheat flour became (0.792 mg/100 g).

2.7.5. Sodium and Potassium:

Sodium and Potassium are important vitamins; their deficiency is rare, immoderate consumption of Sodium might also additionally result in hypertension. Potassium regulates heartbeat, keeps fluid stability and enables muscular tissues contraction. Lovis (2003) pronounced values of Sodium of teff grain as (471 mg/100 g), wheat flour (2mg/100 g) and values of (401 mg K/100 g) for teff grain whilst wheat flour of (100 mg K/100 g). Melak (1966) pronounced values (360 mg K/100 g and 200 mg K/100 g) for two teff cultivars, whilst values (330 and 440 mg K/100 g) for two wheat cultivars, additionally in line with the equal authors' values were (22.00 mg Na/100 g) and (21.22 mg Na/100 g) for two teff cultivars, whilst values (16.85mg Na/100 g) and (39.20 mg Na/100 g) for two wheat cultivars.

2.7.6. Lead

Lead is harmful to the nervous system and causes blood disorders (Morgano, 2011). Lead toxicity influences brain, heart, kidneys, liver, nervous system, and pancreas. It may cause many signs and symptoms such as abdominal pain, anemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, fatigue, headaches and hypertension. It also diminishes IQ in children (Taher, 2003).

2.7.7. Cadmium

Cadmium has no known nutritional value, and it has been considered an extremely significant pollutant affecting all life forms because of its high toxicity and great solubility in soil and water and easy accumulation in roots of most plant tissues. Excessive Cd exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects, and cancer (Nordberg, 2003).

3. MATERIALS AND METHODS

3.1. Description of the study area

3.1.1. Geographical location

Enemay is one of the woreda found in Amhara region of Ethiopia. It is located at Eastern Gojam zone about 265 km from Bahir Dar and lies between $10^0$39'59.99'' latitude and $32^0$88'0.00'' longitudes. The annual mean temperature of the area ranges between 15.1-27.5 ^{0}C and the annual mean rainfall ranges 1201-1800 mm. Enemay is bordered on the south by Dejen, on the West Debaytelatgen, on the north by Enargenawga and on the East by Shebelberenta. The administrative center of this wereda is Bichena (Enemay woreda administration office).

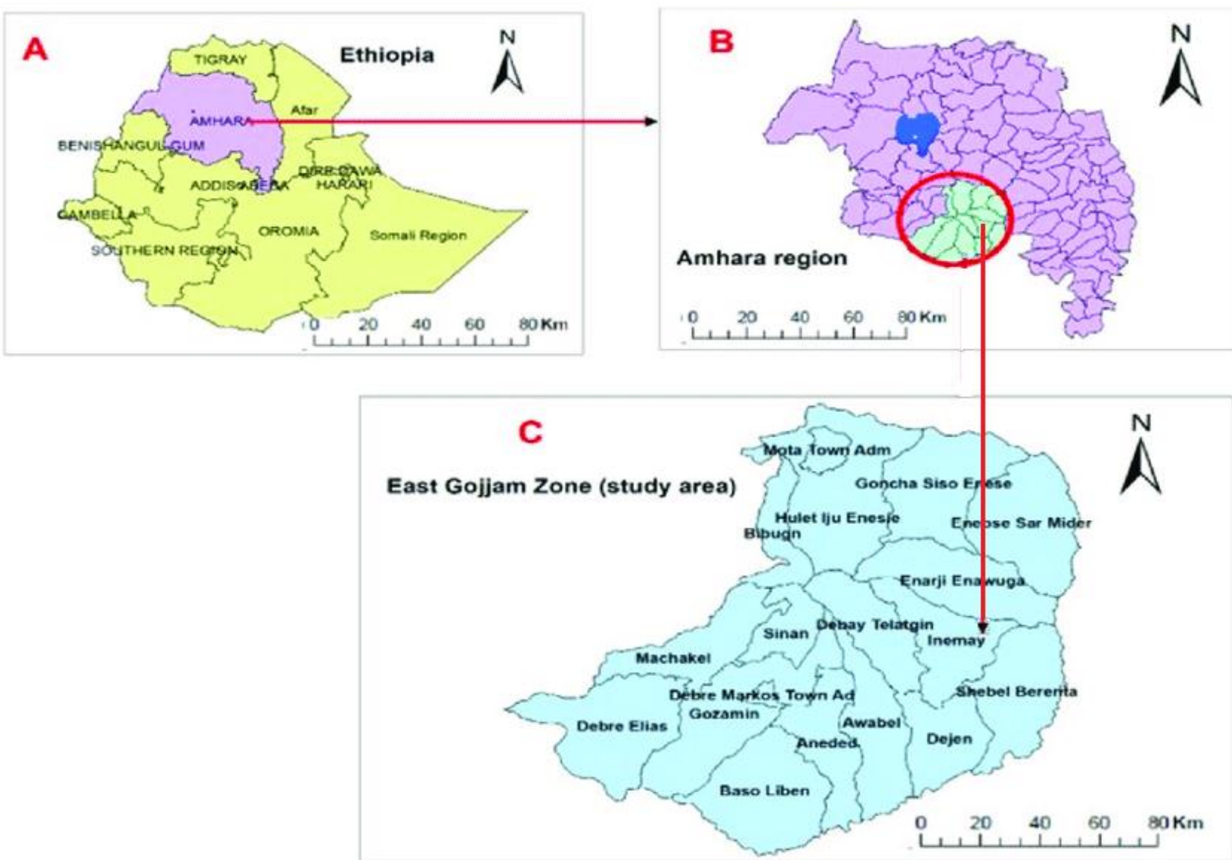


Figure 3.1: Study area location map of East Gojjam zone, Enemay woreda

3.2. Chemicals and reagents

HNO_3 (69-70%, Supreme Enterprises Cantt, India) and H_2O_2 (30%, Scharlau, European Union) were used for the digestion of teff samples. An intermediate standard solution of 10 mg/L for each studied metal was prepared from a 1000 mg/L stock solution of the respective metal from which the calibration standards of each metal were prepared.

3.3. Instrument and apparatus

Digital analytical balance (Mettler Toledo, Model At250 and Switzerland) for weighing the teff samples, Oven for drying the samples, mortar and pestle for grinding and homogenizing the teff samples, hot plate (Gallenhamp, England) to digest the teff samples, refrigerator (Hitachi, Tokyo, Japan) to preserve the solutions until analysis, and Inductively Coupled plasma Optical Emission Spectrometer (PerkinElmer Optima8000) to determine the studied metal concentration.

3.4. Procedure

3.4.1. Sampling of red teff

Red teff samples were collected from farmers of Enemay wereda in each of five kebeles Mankorkoya, Bechenadebir, Yikebehana, Yerese and Debissa. About 50 g of each sample was collected with polyethylene plastic bags randomly totaling three bags per sample then, pooled together and homogenized. Per each sample, a total of 150 g was collected from one kebele . About 50 g of the sample was taken to laboratory for analysis from each kebele.

3.4.2. Sample preparation

Light and heavy impurities were removed from sample by sieving, sifting, winnowing. Next, the samples were washed with tap water to remove all dust particles and by distilled water and kept in an oven and dried at 105 ^{0}C for 5 hours. The dried samples were grind by using mortar and pestle, and sieved using a sieve. Then the dried powdered teff samples were kept in polyethylene bottle for sample digestion.

3.4.3. Digestion of red teff samples

The powdered red teff sample were digested by optimized procedure of 3 mL of HNO_3 (69-70%) and 2 mL of H_2O_2 (30%) for 2:30 hours and digestion temperature of 120 ^{o}C. the digested solution was allowed to cool and 20 mL distilled water was add then filtered with filter paper (42 mm) in to 50 mL volumetric flask after that distilled water was added to fill until the mark.

Finally the digested samples were placed in refrigerator until analysis using ICP - OES. Digestion of a reagent blank was also performed in parallel with red teff samples keeping all digestion parameters the same.

3.5. ICP-OES Instrumentation

The concentrations of metals (Mg, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb) in red teff samples were determined by PerkinElmer Optima 8000 ICP-OES instrument equipped with WinLab32^{TM} 5 - 5 -1 of ICP Version 4.0 software for simultaneous measurement of all analyte wavelengths of interest (Table 2). specific working parameters were set as follows: RF power (W) of 1500 Watts, Plasma gas flow rate (L/min) of 10 L/min, auxiliary gas flow rate of 0.3 L/min, nebulizer gas flow rate of 0.7 L/min and sample flow rate of 1 mL/min. samples were analyzed three times for each metals.

Figure 3. 2: Optima 8000 ICP-OES

Table 1: Emission wave length of the analyzed metals using ICP-OES.

Metals	wave length
Mg	285.2 nm
Cr	267.7 nm
Mn	257.6 nm
Fe	238.2 nm
Ni	231.6 nm
Cu	327.4 nm
Zn	206.2 nm
Cd	228.8 nm
Pb	220.4 nm

3.6. Method Validation

3.6.1. Precision and Accuracy

Accuracy is how close the measured value is agreed to measure value. The accuracy of the optimized procedure was evaluated by spiking experiments. Accuracy was expressed as the percent recovery of an analyte that has been spiked to the samples in a known concentration before digestion and subsequent analysis. The acceptable ranges of percentage recovery for the studied metals will be within 80 –120 % for metal analysis (Abebaw; Ararso, 2013).

The precision of an analytical procedure describes the closeness of individual measures of an analyte among series of measurements. It is usually expressed as the variance (s^2), standard deviation (SD) and relative standard deviation (RSD) of replicate measurements (Bhagwan; Birhanu, 2015).

3.6.2. Method detection limit (MDL)

Method detection limit is the smallest mass 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. The limit of detection is most commonly defined as the amount of analyte that gives a signal equal to three times the standard deviation of a blank (Mitra, 2003). In this study, after digestion of the blank solutions containing HNO_3 and H_2O_2 doublet readings was taken for each blank and the standard deviation was calculated. The MDL of each element was obtained by multiplying the standard deviation of the reagent blank by three (MDL = 3×δ blank, δ blank is standard deviation of the blank readings) as indicated in table 2.

3.6.3. Method Quantitation limit (MQL)

The lowest concentration level at which a measurement is quantitatively meaningful is called the limit of quantitation (MQL).The MQL is most often defined as 10 times the signal/noise ratio if the noise is approximated as the standard deviation of the blank ,the MQL is 10 × δ of the blank (Mitra, 2003). In this study, MQL was obtained from doublet analysis nine reagents blanks which were digested in the same digestion procedure for powder red teff samples. The MQL was calculated by multiplying standard deviation of the reagent blank by ten (MQL = 10 × δ blank) as indicated in table 2.

Table 2: Instrument detection limit (IDL), method detection limit (MDL) and method quantitation limit (MQL) for the studied metals.

Metal	IDL (mg/kg)	MDL (mg/kg)	MQL (mg/kg)
Mg	0.030	0.096	0.32
Cr	0.007	0.0234	0.078
Mn	0.001	0.009	0.03
Fe	0.005	0.0708	0.236
Ni	0.015	0.021	0.07
Cu	0.008	0.027	0.09
Zn	0.006	0.03	0.1
Cd	0.003	0.03	0.1
Pb	0.042	0.12	0.4

IDL = instrument detection limit; MDL = method detection limit and MQL method quantification limit

3.6.4. Recovery

Analytical method is suitable when it's validated for its intended purpose. Due to the absence of certified reference material for the samples within the laboratory, the optimized digestion procedure was validated by spiking the samples with a typical of known concentration of the analyte metals and therefore the efficiency of the optimized procedure was checked. This was done by adding a Known amount of standard metal solutions to the red teff samples. The spiking procedure was performed as follows: 200 mg/kg standard of each metal were spiked at once in to a beaker containing 0.5 g of red teff sample and digested with non-spiked samples correspondingly with the sample blank, finally diluted and analyzed for each metals of interest. As indicated in table 3 the results of percentage recoveries for the studied metals in red teff samples were within the acceptable range (80 – 120%). Therefore, the optimized digestion procedure was valid for red teff sample analysis.

Table 3: Summary of the recovery results for spiked standards from red teff samples.

Metal	unspiked sample (mg/kg)	Added amount (mg/kg)	Amount detected in the spiked sample (mg/kg)	Recovery (%)
Mg	1,440	200	1,605	83
Cr	7.7	200	186.4	89
Mn	58.7	200	234.3	88
Fe	256	200	426	85
Ni	11	200	212.0	101
Cu	6.5	200	225.8	109
Zn	64	200	303.4	120

The percentage recovery of the metals was calculated by using the following equation (Mihret2018).

$$\%R = \frac{Spiked\ Sample - Unspiked\ Sample}{Added\ amount} \quad x100$$

3.7. Statistical Analysis

The experiment was performed in doublet (n = 2). The results are reported as the mean ± standard deviation (SD) in dry weight and statistically evaluated by one-way analysis of variance (ANOVA). Subsequently, Tukey's HSD test at 95% level of confidence was applied to identify differences between mean concentrations of metal in all sites. Significance was accepted at 0.05 level of probability ($p < 0.05$) (Muhammad *et al.*, 2012).

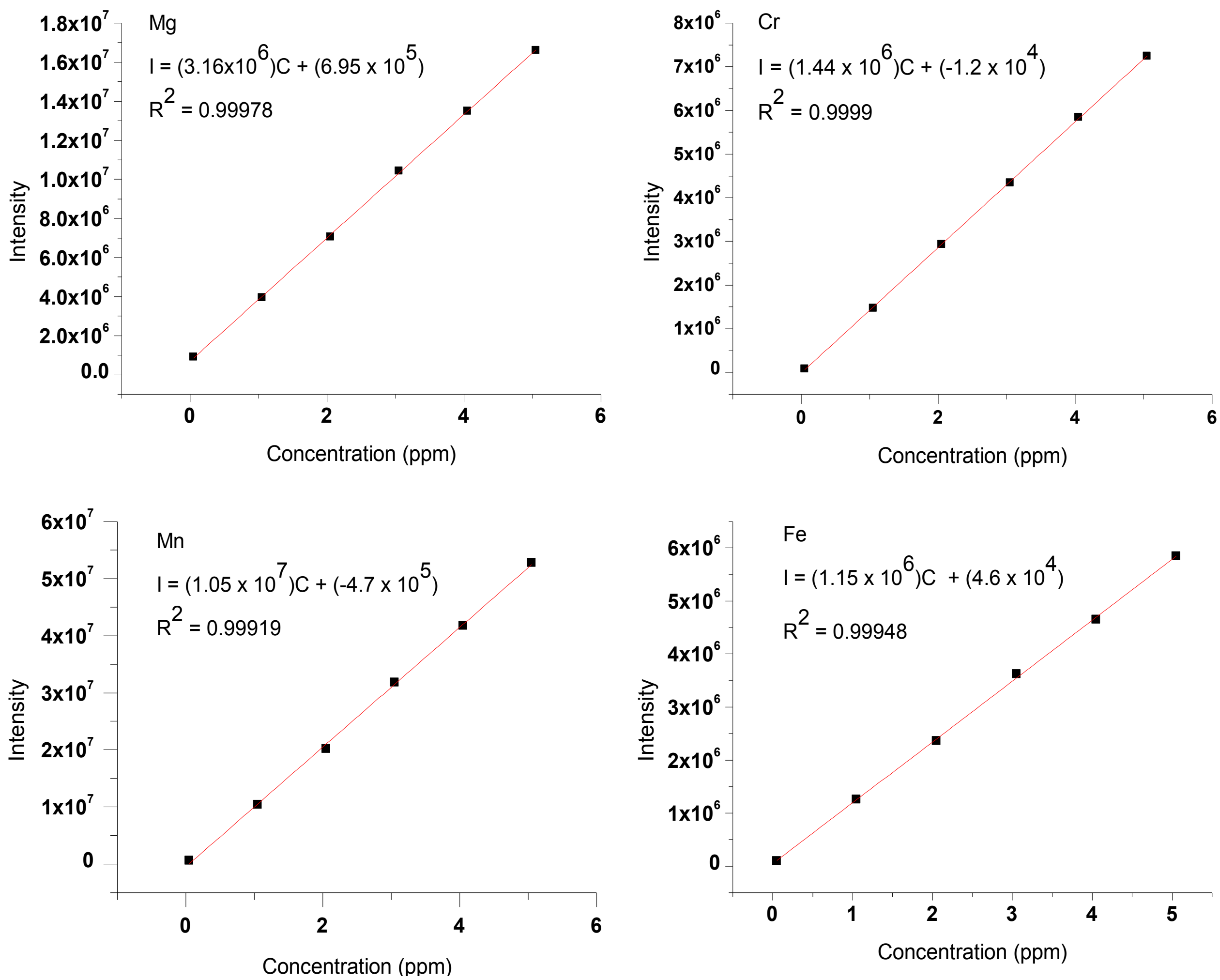
4. RESULT AND DISCUSSION

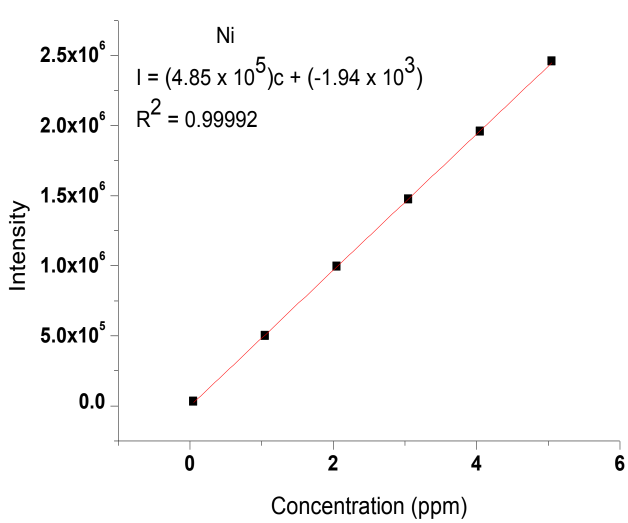
4.1. Instrument calibrations

Intermediate standard solutions of each metals (Mg, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb) containing 10 mg/L were prepared in 100 mL volumetric flask from the standard stock solutions that contained 1000 mg/L. The intermediate standards were diluted freshly with distilled water to the mark to obtain six working standards of each metal of interest for calibration purpose (Abebaw; Ararso, 2013). The instrument was calibrated using six series of working standards and value of correlation coefficient to obtained from intensity verses concentration calibration curve for each metals as indicated in Table 4.

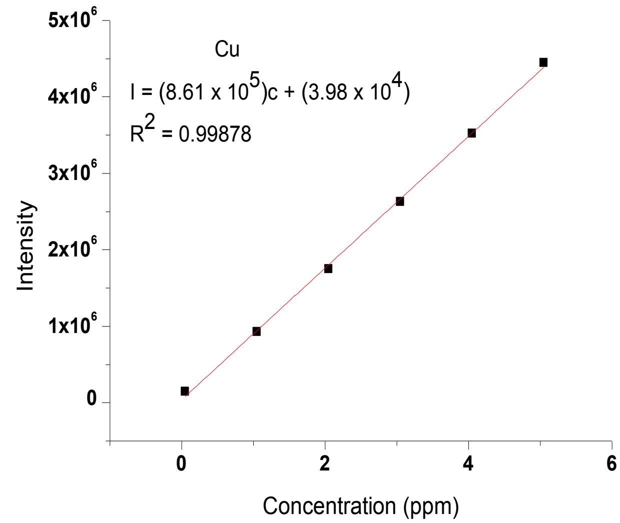
Table 4: Series of working standards and correlation coefficients of the calibration curves for determination of metals in red teff sample using ICP – OES.

№	Metals	Concentration of intermediate standard (mg/L)	Concentration of working standards (mg/L)	determination Coefficient (R^2)
1	Mg	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.99978
2	Cr	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.9999
3	Mn	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.99919
4	Fe	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.99946
5	Ni	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.99992
6	Cu	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.99878
7	Zn	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.99665
8	Cd	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.99956
9	Pb	10	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.9995

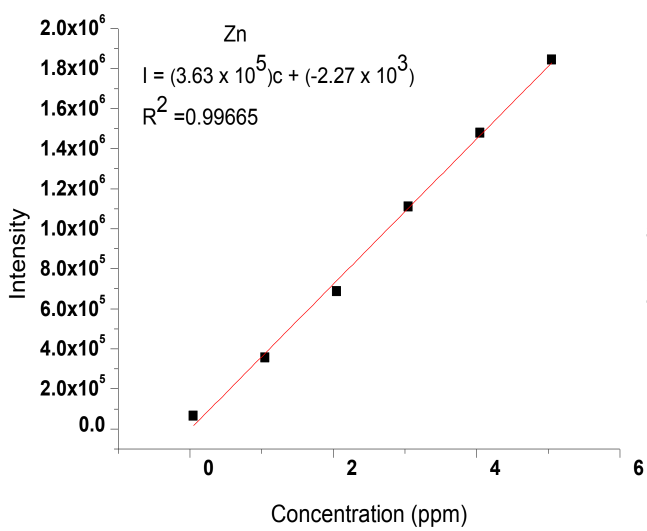

Mg
I = (3.16x10^6)C + (6.95 x 10^5)
R^2 = 0.99978
Intensity
Concentration (ppm)
1.8x10^7
1.6x10^7
1.4x10^7
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
0
2
4
6
Cr
I = (1.44 x 10^6)C + (-1.2 x 10^4)
R^2 = 0.9999
Intensity
Concentration (ppm)
8x10^6
7x10^6
6x10^6
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
0
2
4
6
Mn
I = (1.05 x 10^7)C + (-4.7 x 10^5)
R^2 = 0.99919
Intensity
Concentration (ppm)
6x10^7
5x10^7
4x10^7
3x10^7
2x10^7
1x10^7
0
0
2
4
6
Fe
I = (1.15 x 10^6)C + (4.6 x 10^4)
R^2 = 0.99948
Intensity
Concentration (ppm)
6x10^6
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
0
1
2
3
4
5



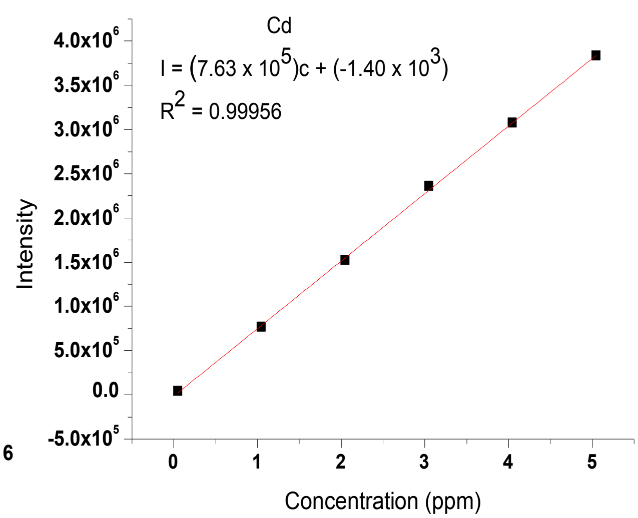
Ni
I = (4.85 x 10^5)c + (-1.94 x 10^3)
R^2 = 0.99992
Intensity
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
0
2
4
6
Concentration (ppm)



Cu
I = (8.61 x 10^5)c + (3.98 x 10^4)
R^2 = 0.99878
Intensity
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
0
2
4
6
Concentration (ppm)



Zn
I = (3.63 x 10^5)c + (-2.27 x 10^3)
R^2 =0.99665
Intensity
2.0x10^6
1.8x10^6
1.6x10^6
1.4x10^6
1.2x10^6
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
0
2
4
6
Concentration (ppm)



Cd
I = (7.63 x 10^5)c + (-1.40 x 10^3)
R^2 = 0.99956
Intensity
4.0x10^6
3.5x10^6
3.0x10^6
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
-5.0x10^5
0
1
2
3
4
5
Concentration (ppm)

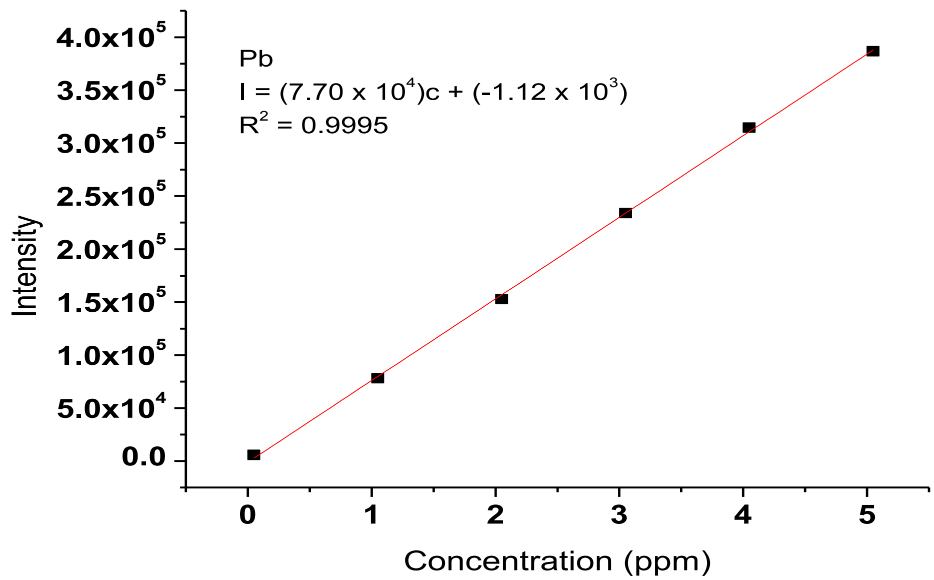


Figure 4. 1: Plot of emission intensity as a function of the concentration for all the studied metals Mg, Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb,

4.2. Optimization of digestion procedure of red teff samples

Optimum working procedure has been developed based on Zeleke,2019 a reliable result. To prepare a clear colorless sample solution the sample digestion procedure were optimized using HNO_3 and H_2O_2 mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature as indicated in Table 5. From the optimization procedures, the mixture of 3 mL HNO_3 (69 - 70%) and 2 mL H_2O_2 (30%), digestion time of 2:30 hours and digestion temperature of 120^{o}C were found to be the optimal conditions. These optimum conditions were selected based on clarity of digests, minimum reagent volume consumption, minimum digestion time, simplicity and minimum temperature applied for complete digestion of sample. The samples were digested using hot plate by taking 0.5 g powdered teff sample.

Table 5: Different conditions tested for optimization of digestion procedure for 0.5 g red teff samples.

No	Weight of sample (g)	Reagent volumes (mL)			Temperature (°C)	Time (min)	Result
		HNO_3	H_2O_2	Total volume			
I. Optimization for reagent volume							
1	0.5	4	1	5	150	2:40	deep yellow
2	0.5	5	1	6	150	2:40	Light red
3	**0.5**	**3**	**2**	**5**	**150**	**2:40**	**Color less (clear)**
4	0.5	3	1	4	150	2:40	Light yellow
5	0.5	2	1	3	150	2:40	Deep red
II. Optimization for temperature							
1	0.5	3	2	5	80	2:40	Deep yellow
2	0.5	3	2	5	100	2:40	Light yellow
3	**0.5**	**3**	**2**	**5**	**120**	**2:40**	**Color less (clear)**
4	0.5	3	2	5	140	2:40	Color less (clear)
5	0.5	3	2	5	150	2:40	Color less (clear)
III. Optimization for digestion time							
1	0.5	3	2	5	120	1:30	Deep yellow
2	0.5	3	2	5	120	2:00	Light yellow
3	**0.5**	**3**	**2**	**5**	**120**	**2:30**	**Color less (clear)**
4	0.5	3	2	5	120	2:45	Color less (clear)
5	0.5	3	2	5	120	3:00	Color less (clear)

4.3. Concentration of metals in red teff sample

Table 6, show the mean values of the analyzed metals determined from triplicate analysis of the samples using ICP - OES and the results are reported in terms of mean ± SD for all the metals. Among all the determined metals the highest concentration of metal analyzed was Fe with concentration 2,341 ± 10.4 mg/kg from Yikebhana followed by Mg with concentration

1,805 ± 15 mg/kg from Yikebehana and Mn with concentration 139 ± 0.6 mg/kg from both Bechenadebir and Yikebhana. However, the concentration of Cd and Pb were not detected in all samples.

Table 6: A Metal concentrations (mg/kg) in the red teff samples.

Metals	N	Sampling site									
		Yerese		Debisa		Bechenadebir		Mankorkoya		Yikebehana	
		mean ± SD	% RSD	mean ± SD	% RSD	mean ± SD	% RSD	mean ± SD	% RSD	mean ± SD	% RSD
Mg	2	1,600 ± 13.2	0.8	1,432 ± 4.4	0.3	1,786 ± 7.9	0.4	1,454 ± 118	8	1,805 ± 15	0.8
Cr	2	14.7 ± 0.5	3.4	1.2 ± 0.1	8.3	26.3 ± 0.6	2.3	13.7 ± 0.5	3.6	41.9 ± 0.4	0.9
Mn	2	66.7 ± 0.8	1.2	94.7 ± 0.5	0.5	139 ± 0.6	0.4	49.4 ± 0.0	0	139 ± 0.6	0
Fe	2	239 ± 2.4	1.0	167 ± 1.9	1	544.5 ± 3.2	0.5	239 ± 3.4	1.4	2,341 ± 10.4	0.4
Ni	2	11 ± 0.3	2.7	8.6 ± 0.2	2.3	18.3 ± 0.3	1.6	9.8 ± 0.1	1	24.3 ± 0.4	1.6
Cu	2	6.5 ± 0.1	1.5	21.3 ± 0.1	0.5	10.6 ± 0.1	0.9	6.0 ± 0.1	1.7	9.4 ± 0.1	1
Zn	2	47.3 ± 0.4	0.8	73.5 ± 0.7	0.9	27.5 ± 0.3	1	62.9 ± 0.8	1.2	37.1 ± 0.9	2.4
Cd	2	ND		ND		ND		ND		ND	
Pb	2	ND		ND		ND		ND		ND	

ND = not detected

As indicated in Table 7, the highest concentration of Fe recorded in the sample from Yekebehana (2,341 ± 10.4 mg/kg) than another four sampling site, this may be due to the difference in mineral contents of soil or pH of soil. The average concentration of Mg metal in red teff samples being analyzed in all five samples was 1,615 ± 31.7 mg/kg. As a result Mg was observed to be the highest of all analyzed metals. This maximum value compared with other mineral nutrients indicated that Mg is the major nutrient component of red teff. The higher levels of magnesium in red teff are probably due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue (Chandravanshi, 2010). The other probable reasons for a higher concentration of Mg is if the soil that has been used for cultivating the plant, are highly fertilized with manure and organic residues, they were high in available potassium, calcium and magnesium. Hence, the plant has high amount of this metal (Watson and Wettasingha, 1977).

From the level of essential trace metals the average concentration of Fe (706 ± 4.3 mg/kg) was the most accumulated trace metal followed by Mn (97.8 ± 0.5 mg/kg) and Zn (49.7 ± 0.6 mg/kg) while Cu (10.8 ± 0.1 mg/kg) took the lowest level in red teff samples. The highest concentration of Fe may be attributed to its higher levels in the soil (Birhanu and Chandravanshi, 2015).

In this study, the average concentration of metals in the red teff samples from the five kebeles decreased in the order Mg (1,615 ± 31.7 mg/kg) > Fe (706 ± 4.3 mg/kg) > Mn (97.8 ± 0.5 mg/kg) > Zn (49.7 ± 0.6 mg/kg) > Cr (19.6 ± 0.4 mg/kg) > Ni (14.4 ± 0.3 mg/kg) > Cu (10.8 ± 0.1 mg/kg) but the non-essential metal Pb and Cd were not detected at all. The range of concentration (in mg/kg) pattern of metals in red teff sample collected from five sampling sites are given in Table 7.

Table 7: Range of mineral nutrient concentrations in red teff samples.

Metal	Concentration range (mg/kg)	Metal	Concentration range (mg/kg)
Mg	1,432 - 1,830	Ni	8.6 - 24.3
Cr	1.2 - 41.9	Cu	6.0 - 21.3
Mn	49.4 - 139	Zn	27.5 - 73.5
Fe	167 - 2,341		

The graph in Figure 4.2 and 4.3 indicates, the distributions of metals in all the studied farm lands were widely varied. This may be due to the difference in mineral contents of soil, pH of soil, and modern large scale agricultural activities, i.e. use of different types of fertilizers, pesticides, herbicides and other chemicals (Adane and Chandravanshi, 2017). Differences in the environmental conditions and crop inherent potential to take nutrient from the soil resulted in the differences on their metabolic activities like accumulations of different metals in their body (Ncube *et al*, 2012).

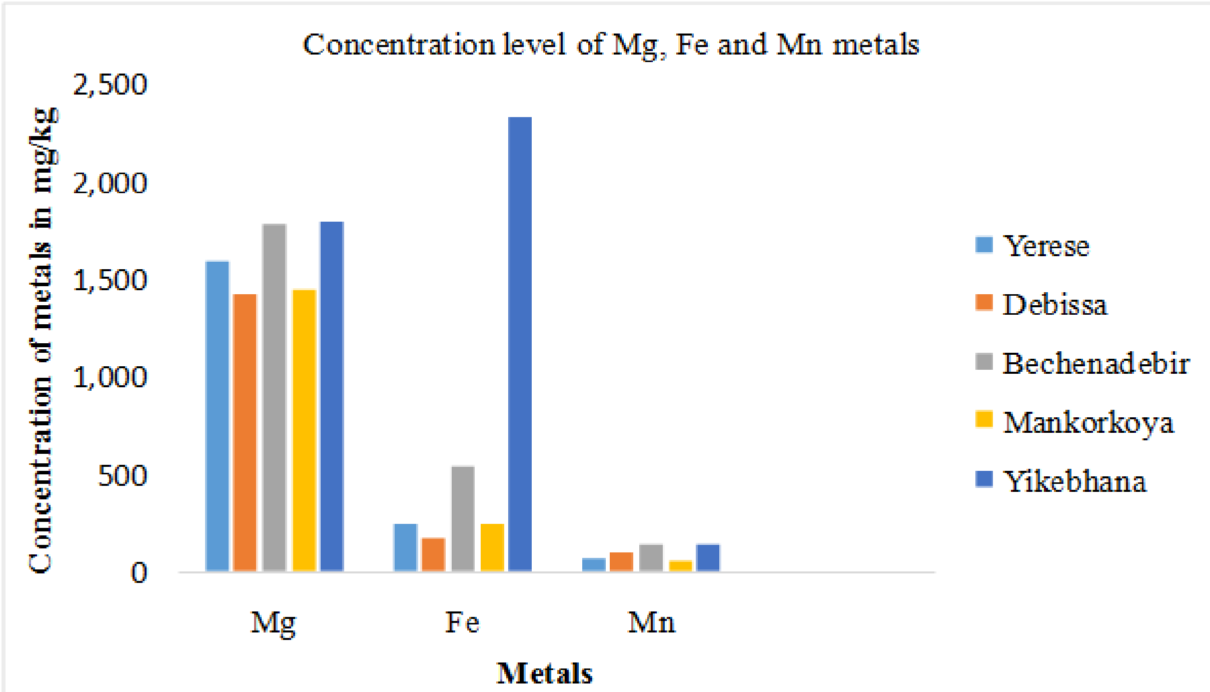


Figure 4. 2: Distribution pattern of Mg, Fe and Mn in the red teff sample.

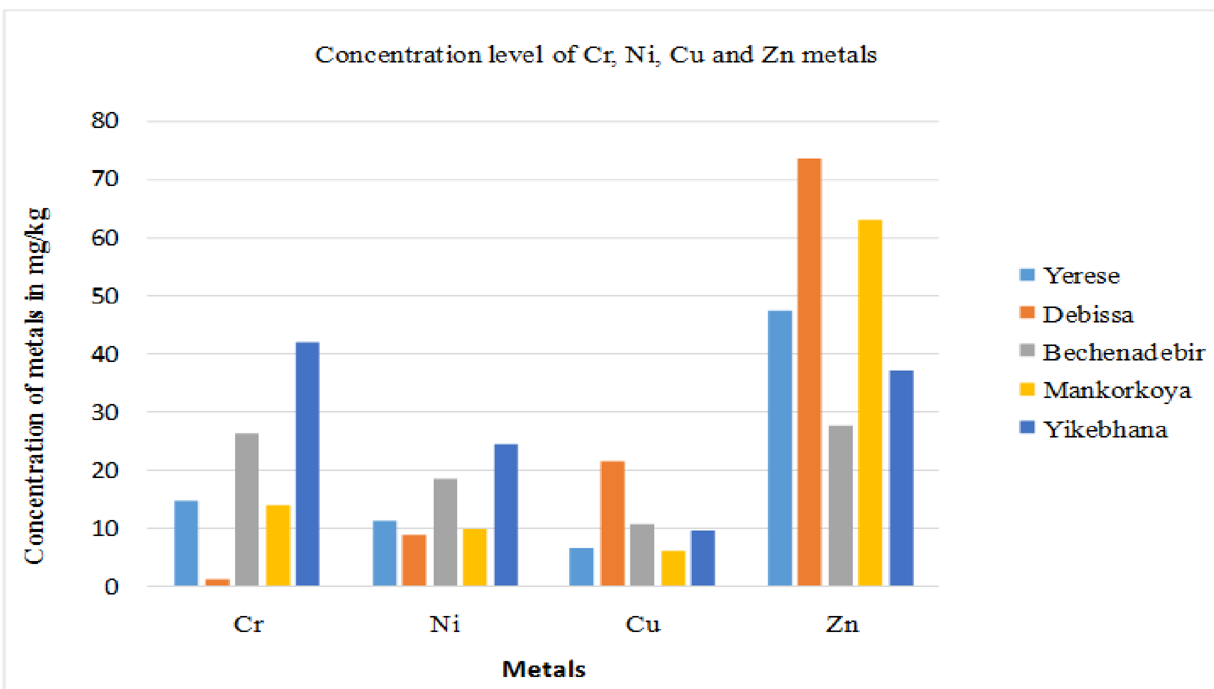


Figure 4. 3: Distribution pattern of Cr, Ni, Cu and Zn in the red teff sample.

4.4. Comparison of the Mineral Nutrient Contents of red teff with other teff verities

As shown in Table 8 the result of Mn found in this study is higher than the value of results cited for white teff by Zeleke, 2009. On the other hand, the value of Mn in this study is also lower than the value found in mixed and red teff. The values of Mg and Zn are comparable with values for red teff as suggested by zeleke, 2009. While the value of Cu in this study is similar with the value obtained by Zeleke, 2009 in white teff. The level of Mg and Cu determined in this study is lower than the value reported in A. Habte *et al.,2020* for red and white teff. But the values of zinc are comparable with values for red and white teff as suggested by A. Habte *et al.,2020*. The level of Fe determined in this study is lower than the value reported in A. Habte et al.,2020 and Zeleke, 2009 for red mixed and white teff .This may be due to the difference in mineral contents of soil, pH of soil, and modern large scale agricultural activities.

Table 8: Comparison of studied metal concentrations (mg / kg, dry mass) in red teff with reported values

Type of teff	Metals concentration (mg/kg)							Reference
	Mg	Cr	Mn	Fe	Ni	Cu	Zn	
White teff	1,532	NR	48.4	160	NR	10.8	3.85	Zeleke,2009
Mixed teff	1,737	NR	132.6	201	NR	16	8.54	Zeleke,2009
Red teff	1,619	NR	224	246	NR	25	49	Zeleke,2009
White teff	2475	NR	68	212	NR	25	53	A. Habte *et al.,2020*
Red teff	2552	NR	75	229	NR	22	57	A. Habte *et al.,2020*
Red teff	1,615	19.6	97.8	706	14.4	10.8	49.7	Present study

4.5. Comparison of Individual Metals Status

Magnesium

Magnesium is the first most accumulated essential metal in the red teff. The mean concentration of Mg in red teff sample ranged from 1,432 ± 4.4 mg/kg to 1,805 ± 15 mg/kg (Table 6). Which is in agreement with the range of values (1531.6 ± 94.5 to 1737 ± 88.9 mg/100kg) reported by Zeleke, 2009 and the value (1840 mg/kg) reported by USDA, 2016. The highest and the lowest concentration of Mg is recorded in the sample from Yekebehana (1,805 ± 15 mg/kg) and Debissa (1,432 ± 4.4 mg/kg) respectively. The mean concentration value order of Mg according to the farmlands is as follows: Yekebehana > Bechenadebir > Yerese > Mankorkoya > Debissa. One way ANOVA reveled that there was no significant difference ($p < 0.05$) in levels of Mg across the sites.

Chromium

The Cr content ranged from 1.2 ± 0.1mg/kg to 41.9 ± 0.4 mg/kg. In the present study, the highest concentration of Cr was found in Yekebehana (41.9 ± 0.4 mg/kg) and the lowest Cr content was in Debissa (1.2± 0.3 mg/kg) as shown Table 6.The concentration of Cr in the studied samples is arranged in a decreasing order: Yekebehana > Bechenadebir > Yerese > Mankorkoya > Debissa. One way ANOVA reveled that there was significant difference ($p < 0.05$) in levels of Cr across the sites.

Manganese

The mean concentration of Mn in red teff ranged between 49.4 ± 0.0 mg/kg to 139 ± 0.6 mg/kg (Table 6). The highest value of Mn was recorded in both Bechenadebir and Yekebehana (139 ± 0.6 mg/kg) and the lowest concentration of Mn was recorded in the sample from Mankorkoya (49.4 ± 0.0 mg/kg). Considering the mean concentration value order of Mn was as follows: Bechenadebir and Yekebehana > Debissa > Yerese > Mankorkoya. One way ANOVA reveled that there was significant difference ($p < 0.05$) in levels of Mn across the sites. The concentration of Mn in this study was below maximum permissible level 500 mg/kg dry weight set by FAO/WHO, 2001.

Iron

The mean concentration of Fe is found to be the highest of all trace essential metals measured in red teff samples. The concentration of Fe in this study ranged from 167 ± 1.9 mg/kg to 2,341 ± 10.4 mg/kg. According to sampling sites mean concentration of Fe was highest in Yekebehana and lowest in Deisbsa respectively (Table 6). One way ANOVA revealed that there was significant difference ($p < 0.05$) in the levels of Fe across the sites.

Zinc

Zinc is the fourth most concentrated metal next to Mg, Fe and Mn in red teff samples analyzed. The levels of Zn ranged between 27.5 ± 0.3 mg/kg to 73.5 ± 0.7 mg/kg in the samples Among all those studied areas the highest level of Zn was determined in Debissa 73.5 ± 0.7 mg/kg and the least in Bechenadebir 27.5 ± 0.3 mg/kg. One way ANOVA revealed that there was significant difference ($p < 0.05$) in the levels of Zn across the sites. The mean concentration of Zn in this study was below maximum permissible level 99.4 mg/kg dry weight set by Girma and Meareg, 2017.

Copper

The mean concentration of Cu in this study ranged from 6.0 ± 0.1 mg/kg to 21.3 ± 0.1 mg/kg.while the value (8.1 mg/kg) reported by (USDA, 2016). The highest and lowest concentration of Cu recorded in the sites of Debissa and Mankorkoya respectively. One way ANOVA revealed that there was significant difference ($p < 0.05$) in the levels of Cu in the sites. The present investigation revealed that the concentration of Cu was below the maximum permissible limit 73.3 mg/kg for human health set by FAO/WHO, 2001.

Nickel

The highest mean concentration of Ni is obtained in Yekebehana (24.3 ± 0.4 mg/kg) and the lowest concentration of Ni was analyzed in Debissa (8.6 ± 0.2 mg/kg). One way ANOVA revealed that there was significant difference ($p < 0.05$) in the levels of Ni across the sites.

5. CONCLUSION

In this study, the concentration of essential metals (Mg, Cr, Mn, Fe, Zn, Cu and Ni) and non-essential metals (Cd and Pb) in red teff samples cultivated in Enemay woreda collected from five different farm lands were determined by ICP - OES. The optimized digestion procedure applied on the samples for the analysis of metal concentration was effective. As it was checked by the recovery experiment; a good percentage recovery was obtained from 80% - 120% for the metal identified.

Among the metals identified Mg was found to be the highest determined metal in the samples of all five sites. Hence, it is the most abundant element for the red teff followed by Fe and Mn. However, the investigated metals, Cd and Pb were not detected at all in the samples of all five sites. Among microelements the concentration of Fe was observed to be the highest in Yekebehana sample followed by Mn in the sample taken from Bechenadebir and Yekebehana. Where as the concentration of Zn was observed to be the third in the sample taken from Debissa.

Mean Concentration of metals in samples of different sites were compared using Tuky HSD and their significance were computed using one way ANOVA. Statistically, significant differences ($p < 0.05$) among the mean concentrations of metals in all red teff samples were observed.

6. RECOMMENDATION

- ✓ Additional investigation on soil pH, nature of agricultural inputs like fertilizers, pesticides, herbicides are also recommended.
- ✓ Government bodies and other stakeholders should create awareness on the use of fertilizers that contain essential nutrients on areas having shortage of minerals nutrients.
- ✓ The relationship between the metal levels of the red teff crop and soil samples is recommended by taking large number of sites and different methods to come up with a comprehensive conclusion.

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1. APPENDIX

Table 9: Statistical analysis of variance (ANOVA) between and within red teff samples at 95 % confidence level.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Mg	Between Groups	374660.267	4	93665.067	32.496	0.000
	Within Groups	28823.333	10	2882.333		
	Total	403483.600	14			
Cr	Between Groups	2820.809	4	705.202	2940.385	0.000
	Within Groups	2.398	10	.240		
	Total	2823.207	14			
Mn	Between Groups	20135.357	4	5033.839	16370.209	0.000
	Within Groups	3.075	10	.307		
	Total	20138.432	14			
Fe	Between Groups	10276521.058	4	2569130.264	92131.955	0.000
	Within Groups	278.853	10	27.885		
	Total	10276799.911	14			
Ni	Between Groups	534.136	4	133.534	248.281	0.000
	Within Groups	5.378	10	.538		
	Total	539.514	14			
Cu	Between Groups	464.233	4	116.058	24012.034	0.000
	Within Groups	.048	10	.005		
	Total	464.281	14			
Zn	Between Groups	4187.021	4	1046.755	2466.823	0.000
	Within Groups	4.243	10	.424		
	Total	4191.264	14			