Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 7, Issue 1, pp: (13-24), Month: January - April 2020, Available at: www.noveltyjournals.com

LEVELS OF SELECTED METALS IN WHITE TEFF GRAIN SAMPLES COLLECTED FROM THREE DIFFERENT AREAS OF ETHIOPIA BY USING MICROWAVE PLASMA ATOMIC EMISSION SPECTROSCOPY (MP-AES)

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Abstract: **Teff *(Eragrostis tef* (Zuccagni)) is an important food security crop in Ethiopia and the East African Highlands. The principal use of teff grain for human food in Ethiopia is in the form of *injera*. The levels of essential and non-essential metals in the white teff grains collected from Bure, Debre Markos and Bahir Dar (Ethiopia) were determined by using microwave plasma-atomic emission spectroscopy (MP-AES). After proper sample pretreatment, 5 mL HNO_3 : 1 mL $HClO_4$, 240 ^oC and 2:30 h were the optimized digestion conditions.**

The overall mean concentrations determined (mg/kg, dry weight) were in the ranges of Al (713-1513) > Fe (252-1195) > Ca (233-348) > Zn (69-102) > Mn (20-45) > Cu (13-15) > Pb (1.8-2.8) > Cd (0.8-1.8). In this study Al was determined but not studied by the other reported literatures. Similarly Cd was detected in this study but not by the other reported methods, this is due to the sensitivity differences of the instruments. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution and the percentage recoveries varied from 92-104%. One way ANOVA indicated that there is no significance difference between the mean concentrations of Cu and Mn among the white teff samples but there is significant difference for the other studied metals at 95% confidence level.

Keywords: **Microwave Plasma Atomic Emission Spectroscopy (MP-AES); Minerals; Optimization; Recovery; White teff grain (*Eragrostis tef* (Zuccagni)).**

1. INTRODUCTION

Teff [*Eragrostis tef* (Zuccagni) Trotter] is one of the major and indigenous cereal crops in Ethiopia, where it is believed to have originated (Grant and Greg, 2006). 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. Teff is most known for its minute seed head, with nearly two million seeds per pod and diameter of 0.7 to 1.0 mm. Teff in Amharic literally means the lost seed because if dropped, it is so easily lost (Ketema, 1997).

In Ethiopia, teff is harvested by hand when the vegetative part of the plant turns yellow. Oxen are used to trample the grass to separate the seed from the rest of the plant (Ketema, 1997). With a population exceeding 109 million people, Ethiopia is the only country in the world where teff is intensely grown and produced for human consumption. Teff is a staple food in Ethiopia, consisting of two-thirds of their cereal diet and is primarily used to make *injera*. Teff can also be combined with other baking flours to produce baked products, such as muffins and cookies. Teff has also been linked to other health benefits, such as anemia due to its exceptionally high Fe content (Coleman, 2012).

Teff can grow in altitudes ranging from sea level to 2800 meter above sea level under different moisture, soil, temperature and rainfall regimes. It grows in dry as well as water-logged soils, can tolerate anoxic situations better than maize, wheat and sorghum and is resistant to many pests and diseases. The straw of teff is also used as forage for livestock as well as to reinforce mud or plasters in construction of houses both in rural and urban areas (Kibatu et al., 2017). In Ethiopia the crop occupies over 2.8 million hectares equivalent to 25-30% of the total area covered by cereals. In terms of production, teff is the dominant cereal by area coverage and second only to maize in production and consumption (Merga, 2018).

Teff is a grain crop endemic to Ethiopia where it has sustained people for many generations. Teff is higher in lysine than all other cereals except oats and rice. Teff also has the highest Fe content and more Ca, Cu, Zn, Al and Ba than wheat, barley and sorghum (Ketema, 1997). Teff is best suited for cultivation in a warm climate with temperatures ranging from 10 to 27 ^{o}C and altitudes of (1,000-2,100 m). However, it can survive harsh environments, such as drought conditions or water-logged soil.

Many Ethiopian people are very comfortable with the taste of teff *injera* than any other food. Nutritionally teff is the most valuable grain in Ethiopia, which is considered an excellent source of fiber, Fe and Ca than other cereal grains (Melaku et al., 2005). Recently there is a growing interest in teff grain utilization because of nutritional merits (whole grain) and free of the protein gluten that make teff an increasingly important dietary component for individuals who suffer from gluten intolerance or celiac disease (Boka et al., 2013). With respect to soil contamination, teff is extremely exposed and complete removal of the extrinsic Fe from teff is not possible (Baye et al., 2013).

Teff is the smallest cereal grain with an average length of around 1 mm (Adebowale et al., 2011). The minuteness of teff grains has nutritional and technological implications. For instance, as teff grains are difficult to decorticate, the cereal is consumed as a wholegrain, improving nutrient intake for consumers. The color of teff can vary from white (ivory) to dark brown (black) depending on the variety. In Ethiopia three major categories can be identified, white (*nech*), red (*quey*) and mixed (*sergegna*). White teff generally grows only in the Ethiopian highlands and requires relatively good growing conditions. This along with its higher consumer preference may justify why white teff is the most expensive type of teff. However in recent years red teff, which is believed to be more nutritious, is also gaining popularity among health conscious consumers in Ethiopia.

The genetic and phenotypic diversity of teff in Ethiopia is a national treasure of potentially global importance. Teff is tolerant to many extreme environmental conditions including water-logging and storage pests. Although teff performs well on various soil types the average grain yield in Ethiopia is about 0.7 tons/ha. The yield of teff is even lower in the drier part of the country. This low yield is attributed to nutrient deficiencies mainly of N and P and to the susceptibility of the crop to lodging at higher N. Interest in teff has increased noticeably due to its very attractive nutritional profile and gluten-free nature of the grain making it a suitable substitute for wheat and other cereals in their food applications as well as foods for people with celiac disease. Many gluten-free products may not meet the recommended daily intake for fiber, minerals and vitamins (Kamila et al., 2018).

Minerals are present in foods at low but variable concentrations and in multiple chemical forms. The role of minerals in food is to provide a reliable source of essential nutrients in a balanced and bio-available form. In cases where concentration and bio-availabilities in food supply are low, fortification has been popular (Miller, 1996).

The mineral contamination of teff is probably due to its small size and suggests increased contact with soil over a larger area (Baye et al., 2014). The contamination of cereal grains in Ethiopia particularly in teff has often been associated with traditional methods of threshing grain under the hooves of cattle. Traditional threshing led to 30-38% increase in Fe content mainly due to soil contamination.

Iron is necessary for red blood cells formation and required for oxygen transport throughout the body. Ca is essential for developing and maintaining healthy bones and teeth, assists in blood clotting, muscle contraction, nerve transmission and oxygen transport. Zinc is essential part of more than 200 enzymes included in the digestion, metabolism and reproduction and wound healing. It plays critical role in immune response and is an important antioxidant. Cu is both an essential nutrient and a drinking-water contaminant. It has many commercial uses. Cu does not appear to be a cumulative toxic hazard for man, except for individuals suffering from Wilson's disease. Cu is not considered to be mutagenic, carcinogenic or affect reproduction. Teratogenicity/embryo toxicity is observed in some animal studies (FAO/WHO, 2011). Cu is essential to normal red blood cells formation and connective tissue formation. Mn is a key component of enzymes systems, support brain function and is required for blood sugar regulation. Mn is naturally occurring in many surface water and groundwater sources, particularly in anaerobic or low oxidation conditions and this is the most important source for drinking-water. The greatest exposure to Mn is usually from food (WHO, 2003).

Aluminium is a major component of the earth's crust. It occurs in the environment in the form of silicates, oxides and hydroxides. It is released to the environment both by natural processes and from anthropogenic sources. Mobilization of Al through human actions is mostly indirect and occurs as a result of emission of acidifying substances to the atmosphere. Al is highly concentrated in soil derived dusts from natural processes, coal combustion and activities as mining and agriculture. Al and its compounds appear to be poorly absorbed in humans. Variability results from the chemical properties of the element and the formation of various chemical species occurs, which is dependent upon the pH, ionic strength, presence of competing elements and complexion agents within the gastrointestinal tract (FAO/WHO, 2011).

Cd is a relatively rare element, released to the air, land and water by human activities. In general the two major sources of contamination are the production and utilization of Cd and the disposal of wastes containing Cd. The Cd uptake by plants from soil is greater at low soil pH. Tobacco is an important source of Cd uptake in smokers (FAO/WHO, 2011). Pb is a classical chronic or cumulative poison. In humans, Pb can result in a wide range of biological effects depending upon the level and duration of exposure. Pb has been shown to be associated with impaired neurobehavioral functioning in children (FAO/WHO, 2011).

The main objective of this study was to determine the selected metal contents in white teff samples collected from three different areas of Ethiopia by using MP-AES method. Teff is generally accepted that the grain is highly nutritious although there is some debate about the precise nutritional value of it (Vinning and McMahon, 2006). Regarding the Fe content some authors have estimated to be high and others reflect this high level is attributed to the dust and dirt that cling to the grain (Grant and Greg, 2006). Whereas other studies revealed that Fe content of teff vary between geographical regions possibly as a result of soil contamination (Saturni et al., 2010; Bokhari et al., 2012). Literature review indicated that the mineral contents of teff flour were determined by using different techniques at different times. But the results reported by the different literatures are controversial and have differences; this may be due to sampling error. Additionally, most of the researches were done by collecting teff flour samples from commercially available mill houses; this may increases the contamination levels of the samples. Therefore collecting teff grain samples and determining the metal contents of it by using the MP-AES is very important.

According to (Berhane et al., 2011) Amhara region is the highest teff producer in Ethiopia. But no reported literatures are present about the levels of metals in teff samples collected from the described sampling areas. Additionally, no researches are done on the analysis of minerals of teff grain using microwave plasma atomic emission spectrometer. Hence, determining the mineral contents of teff samples by using microwave plasma atomic emission spectrometer is a new method in Ethiopia and since the method is multi-element system and cost effective, analyzing mineral contents in teff samples using this instrument is important.

MP-AES is a multi-element analysis method, therefore many elements can be determined within a short period of time compared to other spectroscopic methods.

2. EXPERIMENTAL

INSTRUMENTAL

MP-AES consists of a microwave induced plasma interfaced to an atomic emission spectrophotometer (AES). It is used for simultaneous multi-analyte determination of major and minor elements. MP-AES employs microwave energy to produce a plasma discharge using nitrogen supplied from a gas cylinder or extracted from ambient air, which eliminates the need for sourcing gases in remote locations or foreign countries. Samples are typically nebulized prior to interaction with the plasma in MP-AES measurements. The atomized sample passes through the plasma and electrons are promoted to the excited state. The light emitted electrons return to the ground state light is separated into a spectrum and the intensity of each emission line measured at the detector. Most commonly determined elements can be measured with a working range of low part per million (ppm) to weight percent. MP-AES is a technique comparable to traditional AA and AES but with several potential advantages including lower cost of operation and elimination of the requirement for flammable gasses. All measurements were performed using an Agilent 4200 MP-AES (USA), with nitrogen supplied from an Agilent 4107 nitrogen generator.

3. CHEMICAL REAGENTS AND MATERIALS

CHEMICALS AND REAGENTS

$HClO_4$ (70%) and HNO_3 (69-72%) (Sigma Aldrich Steinleim, Germany) were used for the digestion of samples. The reference standards of the metals were the products of Perkin Elmer (Boston, USA). The stock standard solutions 1000 mg L^{-1} were prepared from the nitrate salts of the metals. The working standard solutions of the metals were prepared freshly from the intermediated standard solutions (100 mg L^{-1}).

MATERIALS

The materials used in the laboratory were sample preparation utilized PVC flasks, polyethylene conical flasks, filter paper, 50 mL volumetric beakers for sample and solution preparation, round bottom flask, ceramic mortar and pestle (USA) for grinding and homogenizing the samples, digital analytical balance (four digit) and Kjeldahl technique (England) for the purpose of digestion.

SAMPLING SITE DESCRIPTION

Three white teff samples were collected from the most teff productive areas of three different localities of Amhara regional state of Ethiopia. Particularly from Debre Markos, Bure and Bahir Dar which are located in the north western part of Amhara regional state. The geographical locations (latitude, longitude and elevation) of sampling sites are described as follows. Bahir Dar is located at latitude of 11^{o}35'37.1" N and longitude of 37^{o}23'26.8" E in the northern hemisphere. Bahir Dar is located at the exit of the Abbay from Lake Tana at an altitude of 1,820 meters above sea level. The city is located approximately 578 km north-west of Addis Ababa. Debre Markos is a city in north-west of Ethiopia. It is located in the Misrak Gojjam Zone of the Amhara administrative region, it is located at a latitude and longitude of 10°20′N 37°43′E coordinates and an elevation of 2,446 meters above sea level. Debre Markos is located approximately 306 km far apart from Addis Ababa. Bure is a town in western Ethiopia located in the Mirab Gojjam Zone of the Amhara region, this town is located at a latitude and longitude of 10°42′N 37°4′E with an elevation of 2091 meters above sea level. Bure is located approximately 414 km far apart from Addis Ababa. The reason for selection of these places was based on the availability of the teff and its popularity in consumption.

SAMPLE COLLECTION AND PREPARATION

White teff samples were collected from different teff bags/containers from the north-western areas of Ethiopia (Bahir Dar, Debre Markos and Bure), which are the most teff productive regional areas. From each sample types around 0.1 kg of sub-samples were collected from different teff containers. A total of around 0.5 kg of teff samples were collected through compositing for each teff samples. The samples were sampled by using auger sampler from the containers. The collected samples were kept in polyethylene bags. The collected teff samples were transported to the laboratory. Some unwanted materials in the teff samples were removed. The samples were washed with tap water and then with distilled water to eliminate adsorbed dust and particulate matters. The samples were then air-dried for seven days to remove moisture. The dried samples were ground by using a machine grinder and sieved to mesh size of 0.5 mm. Then the samples were stored in plastic bags (polyethylene) under airtight conditions until the time of digestion.

OPTIMIZATION OF DIGESTION PROCEDURE

Wet acid digestion is one of the methods that are involved to get free metal ions in dissolved form from complex organic matrix based on changing different digestion parameters like volume ratio of reagents added, digestion temperature and duration of time. One of the wet acid digestions can be carried out by Kjeldahl apparatus in which organic components are assumed to decompose in the form of different gaseous forms and other metallic elements are left in the solution except those easily volatile metals like Hg. Moreover it is assumed that digestion is assumed to be complete if the solution is clear and colorless.

Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 7, Issue 1, pp: (13-24), Month: January - April 2020, Available at: www.noveltyjournals.com

Different digestion procedures were carried out for the teff samples using HNO_3 and $HClO_4$ acid mixtures by varying volume of the acid mixture, digestion time and digestion temperature (Boke et al., 2015). The results are summarized in Table I. Optimized procedures were selected based on the usage of lesser reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests. Based on this fact the optimized digestion conditions for the teff samples in this study were (5 mL HNO_3 : 1 mL $HClO_4$) volume ratio of reagents, 240 ^{o}C digestion temperature and 2:30 h digestion time.

DIGESTION OF SAMPLES

Applying the optimized conditions, 0.5 g of powdered teff samples were transferred into a 250 mL round bottom flask. Then 6 mL of a mixture of HNO_3 (69-72%) and $HClO_4$ (70%) with a volume ratio of 5:1 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus fitted with a reflux condenser by setting the parameters temperature and time. The digest was allowed to cool to room temperature for 10 min without dismantling the condenser and for 10 min after removing the condenser. To the cooled solution 10 mL of distilled water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with filter paper (Whatman 125 mm diameter, Germany) into 50 mL volumetric flask. The round bottom flask was rinsed subsequently with around 5 mL distilled water until the total volume reached around 40 mL. Then finally the solution was filled to the mark (50 mL) using distilled water. The digestion was carried out in triplicate for each sample. Digestion of the blank was also performed in parallel with the teff samples keeping all digestion parameters the same. Then the metal concentrations in the digested sample solutions were determined by using MP-AES as shown in Table I.

TABLE I. Reagent ratios and volumes, temperature and time attempted during optimization of digestion of 0.5 g of teff sample.

Trials	Reagent volume (mL)			Temperature	Time (h)	Results
	HNO_3	$HClO_4$	Total	(^{o}C)		
1	1	1	2	240	2:30	Yellow with suspension
2	2	1	3	240	2:30	Cloudy yellow
3	3	1	4	240	2:30	Nearly colorless
4	4	1	5	240	2:30	Slightly colorless
5	**5**	**1**	**6**	**240**	**2:30**	**Clear colorless***
6	6	1	7	240	2:30	Clear colorless
7	3	2	5	240	2:30	Slightly colorless
8	4	2	6	240	2:30	Nearly colorless
9	4	1	5	240	2:30	Nearly colorless
10	5	2	7	240	2:30	Clear colorless
11	5	1	6	240	0:30	Yellow with suspension
12	5	1	6	240	1:00	Yellow with suspension
13	5	1	6	240	1:30	Cloudy light yellow
14	5	1	6	240	2:00	Light yellow
15	**5**	**1**	**6**	**240**	**2:30**	**Clear colorless***
16	5	1	6	240	3:00	Clear colorless
17	5	1	6	150	2:30	Cloudy yellow with suspension
18	5	1	6	180	2:30	Cloudy yellow with suspension
19	5	1	6	210	2:30	Slightly yellow
20	**5**	**1**	**6**	**240**	**2:30**	**Clear colorless***
21	5	1	6	270	2:30	Clear colorless
22	5	1	6	300	2:30	Clear colorless

*The optimized conditions for the three parameters (reagents volume ratio, time and temperature).

4. DETERMINATION OF METALS IN TEFF SAMPLES

INSTRUMENT CALIBRATION

Calibration standard solutions were prepared for each of the metals which were prepared from the MP-AES standard stock solutions that contained 1000 mg L^{-1}. Intermediate standards were used to obtain five working standards for each metal of interest. Then Fe, Ca, Cu, Zn, Mn, Al, Cd and Pb were analyzed by MP-AES. Three replicate determinations were carried out on each sample. The same analytical procedure was employed for the determination of elements in the digested blank solutions. The correlation coefficient for each metals shows that the change in emission with concentration is in good correlation. Five points calibration curve were established by running series of the prepared working standard solutions for each metal. After calibration the sample solutions were aspirated into the MP-AES instrument and readings of the elemental concentrations was recorded.

METHOD DETECTION AND QUANTIFICATION LIMITS

The limit of detection (LOD) is a measure of how sensitive the analytical method is and is the lowest concentration or weight of analyte that can be measured at a specific confidence level. For the determination of limit of detection of the analytical method (LOD), triplicate eight blanks were prepared in parallel and analyzed for their metal contents. The standard deviation (SD) of the eight blanks was calculated and multiplied by three (LOD = $3SD_b$) to determine the method detection limit. The limit of quantification (LOQ) is the smallest quantity of analyte that can be measured with acceptable accuracy and precision and it is described as ten times of the standard deviation. The method detection limits were low enough (≤ 0.56 mg/kg) to determine the metals in the samples at trace levels. The calibration curves were with good correlation coefficients. The wavelength, method detection and quantification limit, correlation coefficient and calibration curve equations are given in Table II.

TABLE II. The wavelength, method detection and quantification limit, correlation coefficient and calibration curve equations.

Metals	Wavelength (nm)	IDL (mg/kg)	MDL[1] (mg/kg)	MQL[2] (mg/kg)	Correlation coefficient	Calibration curve equation*
Fe	372.0	0.10	0.40	1.30	0.9999	I = 5032C + 81.40
Ca	422.7	0.10	0.56	1.90	0.9992	I = 68218C + 8746.00
Cu	324.8	0.10	0.40	1.30	0.9998	I = 10654C + 9283.00
Zn	213.9	0.10	0.22	0.70	0.9990	I = 2448C - 198.70
Mn	403.1	0.10	0.15	0.50	0.9999	I = 27752C - 255.50
Al	396.1	0.10	0.17	0.50	0.9990	I = 17002C - 25.00
Cd	228.8	0.01	0.02	0.10	0.9996	I = 7863C + 733.90
Pb	405.8	0.01	0.06	0.20	0.9997	I = 2889C + 11.22

* I is the emission intensity and C is the concentration. IDL is the instrument detection limit, [1]Method detection limit, [2]Method quantification limit.

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. The validity of the digestion procedures were assured by spiking the samples with a standard solution of known concentration of the target analytes and the percentage recoveries lies from 92-104%, which were within the acceptable range and are summarized in Table III.

TABLE III. Analytical results for recovery test of teff sample

Metal	Concentration in sample (mg/kg)	% Spiking	Amount added (mg/kg)	Spiked sample (mg/kg)	Recovery (%)
Fe	1195 ± 1.0	20	239	1417 ± 0.8	93 ± 4.0
Ca	348 ± 0.5	25	87	428 ± 2.0	92 ± 3.0
Cu	15 ± 0.4	40	6	20.7 ± 0.3	95 ± 0.5
Zn	85 ± 2.0	30	25	109 ± 1.0	96 ± 0.6
Cd	1.8 ± 0.1	50	0.9	2.73 ± 0.1	103 ± 1.0
Pb	2.1 ± 0.1	40	0.84	2.97 ± 0.5	104 ± 2.0

5. RESULTS AND DISCUSSION

THE CONCENTRATIONS OF METALS IN TEFF SAMPLES COLLECTED FROM THE SAMPLING AREAS

As shown in Table IV the concentrations of the metals were determined by MP-AES and the results were expressed in terms of mean ± SD, which were within the range of mean ± 10 and are within the recommended range. Mean values were determined from triplicate analysis of each sample and triplicate samples were used for each sample site.

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 7, Issue 1, pp: (13-24), Month: January - April 2020, Available at: www.noveltyjournals.com

The maximum mean concentration levels in the white teff samples for elements Al, Fe, Ca, Zn, Mn, Cu, Pb, and Cd are 1232, 1195, 348, 84.8, 28.3, 14.5, 2.1 and 1.8 mg/kg, respectively. Similarly the minimum mean concentration levels in the white teff samples for the elements Al, Fe, Ca, Zn, Mn, Cu, Pb, and Cd are 780, 485, 247, 73.0, 19.9, 12.9, 1.8, 0.8 mg/kg, respectively. The concentrations of the metals showed that the white teff samples collected from the Bure site have higher amounts of Fe, Ca, Cu, Al and Cd compared to that of the red and mixed teff samples collected from it. The red teff samples collected from Debr Markos has higher amounts of Fe, Zn, Mn, Al and Pb compared to that of the white and mixed teff samples collected from it. Similarly the red teff samples collected from Bahir Dar has higher amounts of Fe, Ca, Cu, Zn, Mn, Al and Pb compared to that of the white and mixed teff samples collected from it.

In this study Al was determined in the teff samples but not by the other reported literatures. Al is a major component of the earth's crust and occurs in the environment in the form of silicates, oxides and hydroxides. It is released to the environment both by natural processes and from anthropogenic sources. Mobilization of Al through human actions is mostly indirect and occurs as a result of emission of acidifying substances to the atmosphere. Al is highly concentrated in soil derived dusts from natural processes, coal combustion and activities as mining and agriculture. Al and its compounds appear to be poorly absorbed in humans. Variability results from the chemical properties of the element and the formation of various chemical species occurs, which is dependent upon the pH, ionic strength, presence of competing elements and complexion agents within the gastrointestinal tract (FAO/WHO, 2011).

In this study Cd was detected but not by the other reported methods, this is due to the sensitivity difference of the instruments. Cd is a relatively rare element, released to the air, land and water by human activities. In general the two major sources of contamination are the production and utilization of Cd and the disposal of wastes containing Cd. The Cd uptake by plants from soil is greater at low soil pH. Tobacco is an important source of Cd uptake in smokers (FAO/WHO, 2011).

TABLE IV. Mean concentrations (mean ± SD, n = 3, mg kg^{-1} dry weight) of metals in each sample sites analyzed by MP-AES.

Metal	Concentrations (mg/kg) in (mean ± SD) of metals in the white teff samples from		
	Bure	Debre Markos	Bahir Dar
Fe	1195±1	485±1	645±1
Ca	348±0.5	266±1	247±1
Cu	15±0.4[b]	13±0[b]	13±1[b]
Zn	80±1	85±1	73±1
Mn	20±0.4[a]	21±0.5[a]	28±2[a]
Al	1232±2	792±2	780±1
Cd	0.8±0	1.3.±0	1.8.±0.1
Pb	1.8±0	1.83±0	2.1±0.1

The same letter indicated that the values were not significantly different at $p < 0.05$, according to Duncan's multiple range test.

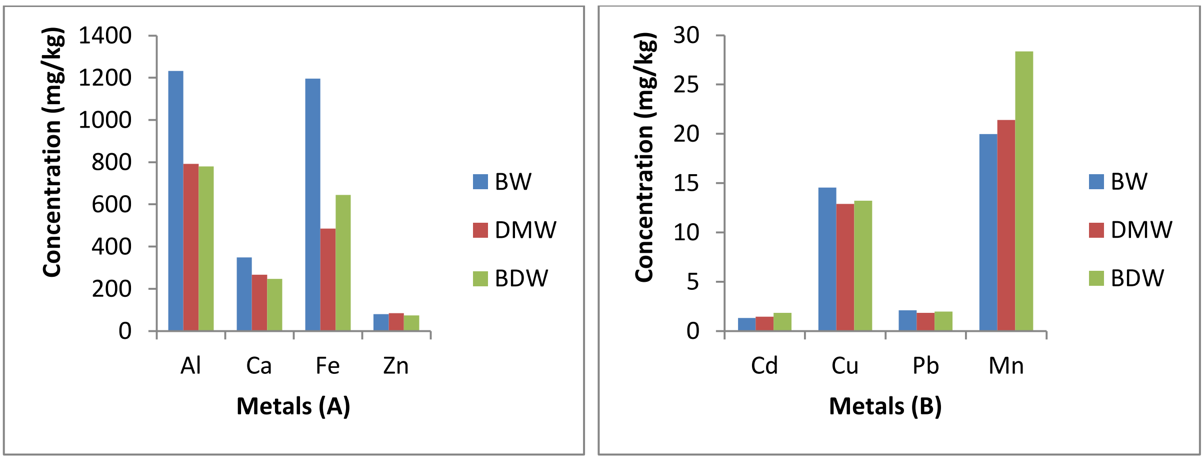

Figure 1. The concentrations of metals (mg/kg) in white teff samples collected from the three sampling areas. (Where: BW = Bure white teff, DMW = Debre Markos white teff and BDW = Bahir Dar white teff).

As shown in Figure 1(A), higher concentrations of Al and Fe are observed in the white teff samples collected from the Bure sampling site, and relatively lower amount of Zn is observed compared to that of Al, Fe, and Ca collected from the three sampling sites. Similarly as shown in Figure 1(B), relatively higher concentrations of Mn and Cu are observed compared to that of Pb and Cd in the white teff samples collected from the three sampling areas. In short as shown in Table 8, the mean concentrations of the metals (mg/kg) in the white teff samples collected from the three sampling areas can be ordered as Al > Fe > Ca > Zn > Mn > Cu > Pb > Cd.

COMPARISON OF LEVELS OF METALS IN TEFF WITH OTHER CEREALS

As can be seen from the Table V, diversified concentration ranges of the studied metals were noticed compared with other cereals like maize, sorghum, wheat and rice. Between teff and wheat, teff and rice, comparable Ca concentrations are observed and between teff and maize comparable Cu concentrations are occurred. For the other metals higher concentrations between teff and the maize, sorghum, wheat and rice with significant differences have been noticed. This variation is may be due to species variability and variations in agricultural practices. The results obtained in this study indicated that Fe, Cu and Zn are more in teff than cereals like sorghum, wheat and rice, Ca is more in teff than maize and sorghum, Ca in teff is comparable with wheat, Cu in teff is comparable with maize.

TABLE V. Comparison of metal contents of teff grain compared to other cereals (mg/kg)

Cereals	Metal concentrations (mg/kg)				Reference
	Fe	Ca	Cu	Zn	
White teff	95-377	170-1240	25-53	24-68	Abebe et al., 2007
Red teff	116-1500	180-1780	11-36	23-67	Gebremariam et al., 2012
Mixed teff	115-1500	788-1470	16	38-39	Zeleke, 2009
Maize	36-48	160	13	26-46	Baye et al., 2014
Sourghum	35-41	50-58	4.1	14-17	Kebede, 2009
Wheat	37	152-395	2.3	17	Abebe et al. 2007
Rice	15	230	1.6	22	Baye et al., 2014
Rice	41-113	198-427	3.3-15	17-140	Tegegne et al., 2017
White teff	485-1195	247-348	13-15	73-85	**This study**

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

As shown in Table VI, the Fe contents determined in teff samples in this study are more than the other reported values and it is within the range of (Baye et al., 2014) study. The amount of Ca determined in this study is relatively lower than the other reported values. Cd is detected in this study but not by the other reported methods, this is due to the sensitivity difference of the instruments. The amount of Cu determined in this method is almost similar with the other reported values. The amount Pb in teff was also determined by (Kibatu et al., 2017) and the values were smaller than the detected values in this study. The Mn determined in this study is less than the reported values of the other studies. The amount of Zn determined in this study is more than the other reported values. Al was determined in this study, but not by the other reported methods.

TABLE VI. Comparison of metals concentration (mg/kg, dry mass) of teff with reported values.

Teff type	Metals concentration (mg/kg)					
	Fe	Ca	Zn	Al	Method	Reference
White	95-377	170-1240	24-68	-	FAAS	Baye et al., 2014
White	160 ± 2	1807 ± 15	30 ± 0.12	-	FAAS	Zeleke, 2009
White	189	1560	-	-	FAAS	Kamila et al., 2018
White	161 ± 2	839 ± 1	27 ± 0.0	-	PTXRF	Kibatu et al., 2017
White	90-146	-	-	-	ICP-OES	Girma et al., 2017
Red	116-1500	180-1780	23-67	-	FAAS	Baye et al., 2014
Red	246 ± 1	1785 ± 10	48 ± 11	-	FAAS	Zeleke, 2009

Mixed	115-1500	788-1470	38-39	-	FAAS	Baye et al., 2014
Mixed	201 ± 1	1686 ± 11	38 ± 0.1	-	FAAS	Zeleke, 2009
Mixed	589	1570	-	-	FAAS	Kamila et al., 2018
Mixed	226 ± 0.02	1162 ± 0.3	34 ± 0.1	-	PTXRF	Kibatu et al., 2017
Mixed	76	1800	36	-	FAAS	Haci Omer, 2018
Mixed	443	-	-	-	PTXRF	Mohammed, 2007
White	**485-1195**	**247-348**	**73-85**	**780-1232**	**MP-AES**	**This study**
Teff type	Cd	Pb	Cu	Mn	Method	Reference
White	-	-	25-53		FAAS	Baye et al., 2014
White	-	-	11 ± 0.1	48 ± 0.04	FAAS	Zeleke, 2009
White	-	-	-	-	-	Kamila et al., 2018
White	-	1 ± 0.0	4 ± 0.03	-	PTXRF	Kibatu et al., 2017
White	-	-	-	-	-	Girma et al., 2017
Red	-	-	11-36	-	FAAS	Baye et al., 2014
Red	-	-	25 ± 0.3	224 ± 0.2	FAAS	Zeleke, 2009
Mixed	-	-	16	-	FAAS	Baye et al., 2014
Mixed	-	-	38± 0.1	133 ± 0.0	FAAS	Zeleke, 2009
Mixed	-	-	-	-	-	Kamila et al., 2018
Mixed	-	1 ± 0.0	4 ± 0.01	-	PTXRF	Kibatu et al., 2017
Mixed	-	-	-	-	-	Haci Omer, 2018
Mixed	-	-	8	-	PTXRF	Mohammed, 2007
White	**0.8-1.8**	**1.8-2.1**	**13-15**	**20-28**	**MP-AES**	**This study**

DAILY RECOMMENDED INTAKE VALUES

Assuming that an average adult person consumes 180 g teff per day on average the amount of mineral intake by the person is shown in Table VII. The amount of Ca that a person can get is lower than the daily recommended values, this indicates that teff alone cannot be a good source of the Ca needed for the daily requirement. Therefore the person must get supplementary Ca from other sources. The amount of Fe is very sufficient for an adult person. The amount of Zn that a man can get from teff is in the range of the daily recommended intake. The values for Al, Cu, Mn, Cd and Pb are all above the allowable limits. Teff in these areas has too much sources of these metals. The man must not consume foods from teff regularly. Since the level of the toxic elements Cd and Pb in the samples are above the allowable limit, it is possible to conclude that, the man is not free from the risks of Cd and Pb as a result of consuming teff, this may be caused due to the contamination of the sample.

TABLE VII. 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.nap.edu, 2015).

Metal	Concentration in teff (mg/kg)	Amount of metal a person can get from 180 g teff (mg)	Recommended daily intake (RDI)	Tolerable upper limit
Fe	485-1195	87-215	9-17 mg	45 mg/day
Ca	247-348	45-63	1000-1200 mg	2500 mg/day
Cu	12.9-14.5	2.3-2.6	0.9-2.3 mg	5 mg/day
Zn	73.0-84.8	13-15	10-20 mg	40 mg/day
Mn	19.9-28.3	3.6-5.0	1.8-2.3 mg	11 mg/day
Al	780-1232	140-222	0.18-1.15 mg/kg b.w/week	1 mg/kg b.w/week
Cd	0.8-1.8	0.14-0.32	0.8 µg/kg b.w/day	7 µg/kg b.w/week
Pb	1.8-2.1	0.32-0.38	0.02-3 µg/kg b.w/day	25 µg/kg b.w/day

ANALYSIS OF VARIANCE (ANOVA)

One way ANOVA was used to compare the mean values of the metals between different sampling sites at 95% confidence level. There were a significant difference among the mean concentrations of Al, Ca, Cd, Fe, Pb and Zn found in the white teff samples collected from the three sampling areas, but no significant difference on the mean concentrations of Cu and Mn among the white teff samples. The presence of significance difference may be due to the presence of different geographical distribution, rainfall, soil composition, harvesting and storing methods.

PEARSON CORRELATION OF METALS

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 metals mean concentrations collected from the three sampling areas of the white teff samples and are summarized in Table VIII. According to this study highest correlations were observed between Ca-Fe, Cu-Fe, Al-Fe, Cu-Ca, Al-Ca, Cd-Ca, Al-Cu, Cd-Cu, Cd-Al and medium and low correlations for the others.

TABLE VIII. Pearson correlation coefficients of the metals in the white teff samples

METALS	Fe	Ca	Cu	Zn	Mn	Al	Cd	Pb
Fe	1.0000							
Ca	0.9232	1.0000						
Cu	0.9767	0.9842	1.0000					
Zn	-0.1203	0.2704	0.0958	1.0000				
Mn	-0.4096	-0.7287	-0.5960	-0.8564	1.0000			
Al	0.9709	0.9884	0.9997	0.1208	-0.6161	1.0000		
Cd	-0.8109	-0.9735	-0.9177	-0.4834	0.8660	-0.9274	1.0000	
Pb	-0.3876	-0.7121	-0.5766	-0.8685	0.9997	-0.5970	0.8538	1.0000

6. CONCLUSIONS

An efficient digestion procedure for the determination of metals in the teff grain samples was optimized and validated through spiking method and a good percentage recovery was obtained for the metals of interest. The mean concentration levels of the metals in the white teff samples collected from the three sampling areas indicated that higher concentrations of Al and Fe were found in the white teff samples collected from Bure sampling area. Similarly, high concentrations of Mn were found in the white teff samples collected from Bahir Dar sampling area than that of Bure and Debre Markos.

The overall mean concentrations determined (mg/kg, dry weight) were in the ranges of Al (780-1232) > Fe (485-1195) > Ca (247-348) > Zn (73.0-84.8) > Mn (19.9-28.3) > Cu (12.9-14.5) > Pb (1.8-2.1) > Cd (0.8-1.8). The results indicated that teff grain is a good source of essential metals but not free from the toxic metals Cd and Pb. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution and the percentage recoveries varied from 92% to 104% which is good and is in the allowed range of 90% ± 10.

The ANOVA results at 95% confidence level suggest that there were no significance difference between the mean concentrations of Cu and Mn among the white teff samples but there were a significant difference for among the other studied metals of the teff samples. Since the levels of the toxic elements Cd and Pb in the teff samples are above the allowable limit, it is possible to conclude that a person who consumes teff produced from this sampling areas is not free from the risks of Cd and Pb, this may be caused due to the contamination of the samples. Uptake of metals by plants is affected by many factors including the soil pH, the prevailing chemical species and the concentration of metals present in the soil.

ACKNOWLEDGEMENTS

I express my gratitude to the Department of Chemistry, Addis Ababa University, Ethiopia, for providing me this study and the laboratory facilities and to the Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia, for sponsoring me this study.

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 7, Issue 1, pp: (13-24), Month: January - April 2020, Available at: www.noveltyjournals.com

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