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LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN THE RAW SEEDS AND PROCESSED FOOD (ROASTED SEEDS AND BREAD) OF MAIZE/CORN (*ZEA MAYS* L.) CULTIVATED IN SELECTED AREAS OF ETHIOPIA

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ABSTRACT. The levels of metals in raw maize seeds and its processed foods (roasted seed and bread) collected from Shendi, Finote Selam and Debre Tabor (Ethiopia) were determined by flame atomic absorption spectrometry (major metals) and graphite furnace atomic absorption spectrometry (trace and heavy metals) after wet digestion. The concentration of the metals determined (mg/kg dry weight) were in the ranges in raw seed, roasted seed, and bread: K 1633–1935, 1753–2008, 1589–2036; Na 125–164, 137–190, 160–195; Mg 388–400, 394–411, 378–401; Ca 66.5–104, 118–178, 102–144; Cr 0.17–1.58, 0.18–1.72, 0.18–1.65; Mn 1.04–3.98, 1.09–4.60, 0.52–2.83; Fe 18.0–115, 16.5–103, 45.3–146; Co 0.41–0.49, 0.50–0.76, 0.34–0.75; Cu 0.04–1.32, 0.04–2.72, 0.05–3.12; Zn 61.7–77.6, 59.2–83.0, 108–116; Pb 0.31–2.59, 0.82–3.11, 1.55–3.41; respectively. Nutritionally, the processed maize foods are found to be better sources of minerals than the raw seeds. Maize seed samples from the three areas of Ethiopia are good sources of essential metals. Analysis of variance indicated significant differences in the levels of all the metals among the three samples means except K, Mg and Pb.

KEY WORDS: Maize (*Zea mays* L.), Seeds, Processed food (Roasted seeds - Kolo, Bread), Ethiopia

INTRODUCTION

Maize/corn belongs to the family Poaceae, genus Zea and species *Zea mays*–corn. Maize/corn is among the most extensively cultivated and consumed cereal crops in the world. It is the main cereal grain as measured by production but ranks third as a staple food, after wheat and rice [1].

More maize is produced, by weight, than any other grain, and almost every country on the Earth cultivates maize commercially for a variety of uses. Currently, the United States, China, Brazil, Mexico, Argentina, India, France, Indonesia, South Africa, and Italy produce 79% of the world's maize production [2].

The large and sudden rise in maize cultivation in some African countries both in terms of yield and in area planted since the 1980s followed the introduction of different new hybrids from the USA and South America. Its taste has been easily accepted by the local population and, therefore, it could rapidly replace traditional starchy foods like sorghum and millets. About two thirds of all African maize is produced in eastern and southern Africa. South Africa is the largest producer of maize followed by Nigeria [3].

Maize is grown globally from 50°N–40°S, and from sea level up to 4000 m altitude. Besides warm sunny weather, corn requires nutrient rich and moist soil. The ideal soil for growing corn is well-drained, preferably a sandy loam. Organic matter such as compost leaves and grass clippings can be added to the soil to improve its overall quality and improve drainage, particularly for heavy clay soil. Like most vegetables, corn grows best in soil with a pH between 5.8 and 6.8. Maize needs soil with high levels of nitrogen for proper growth and development; thus, additional fertilizer can be added at planting or during the growing season. In the cases

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when the pH of the soil is outside the recommended limit, lime or sulfur can be added to adjust the pH to an ideal level for growing corn [4].

Among cereals, maize accounts for the largest share in total production and the total number of farm holdings involved in Ethiopia. In 2010/11, maize accounted for 28% of the total cereal production, compared to 22% for sorghum and 20% for teff, the second and third most cultivated crops in the country. Maize is the largest and the most productive crop in Ethiopia. Ethiopia is the fourth largest maize producing country in Africa next to South Africa, Nigeria and Egypt [3]. In Ethiopia, maize is widely cultivated in areas with altitudes ranging from 1500-2200 meters above sea level.

Currently, maize is produced in Ethiopia using both the traditional practices and extension package. The extension package is of a green revolution type characterized by the use of high yielding seed varieties but it involves the use of fertilizers and chemicals. According to World Bank [5] farmers were only achieving on average 60% of their potential production. The potential for increasing maize production through extensive use of improved seeds is thus high in Ethiopia and is being increased today [6].

Three regional states including Oromia, Amhara and Southern Nations, Nationalities, and Peoples' Region (SNNPR) contribute to 94% of the total annual production. Oromia region alone contributes 60% of the country's maize production. West Gojam is leading producer of maize in the country. East Showa, Jimma, East Welega and West Welega zones are the other major producing zones of maize [5].

Both organic and inorganic fertilizers are being used in the country. Organic fertilizers are farm-generated resources such as crop residues, farmyard manure and legumes. Inorganic (modern) fertilizer is a peculiar input the use of which requires improved varieties of maize seeds. Diammonium phosphate and urea are the two most widely adopted inorganic fertilizers in Ethiopia [5].

In developing countries like African countries, large amount of maize produced in every year is used for the direct human consumption. Industrially maize is refined to generate a wide range of products including corn oil, sweetener, corn starch, and ethanol. New bio-products such as amino acids, antibiotics and degradable plastics are increasingly being synthesized using maize as a raw material. Maize is wet-milled to separate the grain into components (starch, oil, protein and fiber) which are then converted into higher value products [7].

Six major staples: maize, teff, wheat, sorghum, barley and enset (false banana), dominate the national food basket in Ethiopia. Maize is the single most important cereal, accounting for 17% of the per capita calorie intake, followed by sorghum (14%) and teff (11%) [8]. Maize dominates rural consumption baskets, more than that of the urban areas. Maize is an important food security crop in Ethiopia, with the cheapest cost caloric source among all major cereals. Despite having the largest number of livestock in Africa, the use of maize grain as animal feed is very limited in Ethiopia [8].

In Ethiopia, maize is mainly used for food and feed purpose. The stover is used for construction and as a domestic fuel in the rural areas of the country, also as food for cattle and other animals. Though maize is mainly used for human consumption, its share in the total calorie intake in Ethiopia is lower as compared to other African countries [9, 10]. In addition to its usage as food in different forms like bread, roasted seed (called kolo), porridge, *enjera*, and other forms, maize is used as the source of starch for traditional alcoholic beverages production.

The composition of maize endows it with many health benefits. The high fiber content is one characteristic linked to the nutritional benefits of maize. This condition makes it suitable for diets that are made to lose weight and those made with the aim of lowering cholesterol levels. The fiber in whole grains helps to prevent the risk of heart diseases and diabetes, and all its nutrients boost the immune system [11].

Maize, being popular as a food item, is enjoyed by people in various forms, like, whole corn, corn flour, corn starch, corn gluten, corn syrup, corn meal, corn oil, popcorn, cornflakes, etc.

Apart from satisfying the taste buds of its users, maize is also a good source of vitamins, minerals and dietary fiber [7].

Some studies have been conducted on the levels of essential and non-essential metals of maize in Nigeria [12, 13], Brazil [14], USA [14], China [15] and Turkey [16, 17]. Levels essential and non-essential metals in popcorn and cornflake commercially available in Ethiopia have also been reported [18]. The literature surveys revealed that there are no studies conducted on the levels of metals in maize cultivated in Ethiopia.

Since maize is a staple food for many people in Ethiopia, knowledge of its mineral levels is of particular interest. Hence it is crucial to determine the levels of essential and non-essential metals in maize seed cultivated in Ethiopia. The main objectives of this study were to (i) determine the levels of essential and non-essential metals in raw seeds of maize cultivated in selected areas of Ethiopia: Shendi, Finote Selam and Debre Tabor, (ii) to determine the level of essential and non-essential metals in maize seeds processed food (roasted seeds and bread), (iii) to compare the levels of the metals in maize seeds from three sampling areas and (iv) to compare the levels of the identified metals in maize seeds in this study with the literature values.

EXPERIMENTAL

Equipments and apparatuses

Polyethylene bags were used during sample collection and transportation and glass bottles were used while preserving the ground and homogenized samples. Electronic blending device (Foss Knifetec1095, USA) was used for grinding and homogenizing the samples. 250 mL round bottom flasks fitted with reflux condenser were used with Kjeldahl (UK) apparatus hot plate to digest the dried and powdered maize seed samples. Flame atomic absorption spectrophotometer (Analytikjena: Model ZEEnit700P, VGP AAS, Germany) equipped with deuterium arc back ground connectors and hollow cathode lamps with air-acetylene flame was used for the analysis of the analyte metals. Graphite furnace atomic absorption spectrophotometer (PerkinElmer, AAnalyst 600, USA) was also used in this study.

Reagents and chemicals

HNO_3 69-72% (Scharlau Chemie, European Union, Spain), $HClO_4$ 60% (BDH Laboratory Supplies AnalaR®, Poole, England) and extra pure H_2O_2 30% (Scharlau Chemie, European Union, Spain) were used for digestion of the samples. $La(NO_3)_3.6H_2O$ 98% (BDH Chemicals Ltd, Poole, England) was used to minimize the precipitation of Ca and Mg ions in the form of phosphates and sulfates. Stock standard solutions containing 1000 mg/L of the metals K, Na, Mg, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb (BDH Chemicals Ltd Spectrosol®, Poole, England) were used for preparation of calibration standards and for the spiking experiments. Deionized water was used for dilution of samples, intermediate and working metal standard solutions prior to analysis and for rinsing glass wares.

Sampling site description

Debre Tabor. It is a town and a woreda (district) in north central Ethiopia. It is located in the South Gondar zone of the Amhara region, about 100 km southeast of Gondar and 50 km east of Lake Tana. It is 103 km far from Bahir Dar, the capital of the region. According to the road transport authority of Ethiopia, Debre Tabor is about 666 km far from the capital city Addis Ababa. This town has a latitude and longitude of 11°51′N 38°1′E with an elevation of 2,706 meters above sea level [19].

Finote Selam. It is a town and separate woreda in western Ethiopia. It is located in the West Gojjam zone of Amhara region. This town has a latitude and longitude of 10°42′N and 37°16′E with an elevation of 1917 meters above sea level [20]. It is surrounded by Jabi Tehnan woreda. Currently Finote Selam is the administrative center of West Gojjam administrative zone. Finote Selam is about 385 km in northwest direction of Addis Ababa.

Shendi. It is a town in western Ethiopia. It is located in the West Gojjam zone of the Amhara region. This town has a latitude and longitude of 10°38′N and 36°56′E with an elevation of 2,050 meters above sea level [20]. Shendi is the administrative centre of Womberma woreda. Womberma is one of the woredas in west Gojjam zone and is one of the surplus producers of cereal crops in the Amhara region.

Sampling

The possible efforts were made to collect the representative samples of maize seeds from the study areas. About 5 kg of sample was purchased from ten farmers (0.5 kg from each) who were selling maize seed in the open markets in Shendi, Finote Selam and Debre Tabor towns. These ten half kilogram samples from each town were then mixed in to three separate polyethylene plastic bags to get about 5 kg of one bulk sample for each. Then only a representative portion of about 1.5 kg samples from each site were packed in the polyethylene plastic bags and were brought to the laboratory for analysis.

Sample preparation for elemental analysis

The samples were exposed to sun light for drying until constant weight was achieved. A portion of the dried maize seeds were ground using electronic blending device and the powder was transferred to the already prepared plastic bags. Some portions of the samples were roasted using metal pans, get cooled and then got ground using the electronic blending device as it is traditionally done in Ethiopia. This roasted grain in the local term is called “kolo”. The roasted seeds were ground using electronic blending device and the powder was transferred to the already prepared plastic bags.

The bread was prepared according to the traditional procedure used in Ethiopia. From the raw maize flour samples bread was made using clay pans. The flour was mixed well with distilled water. The metal pan was heated and then the prepared dough was placed on the pan. The pan with the dough was covered with lid, and after 10 min the bread was inverted upside down for uniform heat distribution. The bread made was taken off from the pan and allowed to cool. The baked bread was cut in to pieces and exposed to sun light to complete dryness, i.e. until the removal of the water added while baking. The drying process was repeatedly made until a constant weight was obtained. The dried bread sample was crushed in to powder form and made ready in the clean and dry plastic bag until analysis. Similar treatments were made for all the samples from the three sites.

Digestion of the samples

The basic requirements for sample preparation for analysis are to get an optimum condition for digestion. The optimum condition is the one which require minimum reagent volume consumption, minimum reflux time, clarity of digests, and ease of simplicity [21-26]. In this study, to prepare a clear sample solution suitable for the analysis using AAS, a lot of trials of digestion procedures were made on the maize seed powder using the HNO_3, H_2O_2 and $HClO_4$ acid mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature to optimize the digestion procedure (Tables 1 and 2). The acid mixture of

6:2:2 (6 mL HNO_3 (69–70%), 2 mL $HClO_4$ (70%), and 2 mL H_2O_2), digestion time of 3 hours and digestion temperature of 120 °C were found the optimal condition for the digestion of 1 g of samples. These optimum conditions were selected based on clarity of digests, minimum reagent volume consumption, shorter digestion time, and lower temperature applied for complete digestion of the samples.

Applying the optimized conditions 1 g of homogenized dried and ground maize sample was transferred into 250 mL round bottom flasks. 10 mL of the acid mixture with a volume ratio of 6:2:2 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus for 3 hours at the temperature of 120 °C. After 3 hours of digestion time the digested mixture was allowed to cool to room temperature without dismantling the digestion set up. At the time of dismantling the setup, about 10 mL of deionized water was added to the solution and the flask and the lower end of the condenser was rinsed to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering through Whatman 541 filter paper into 50 mL volumetric flask containing about 0.67 g of $La(NO_3)_3.6H_2O$. The round bottom flasks were rinsed subsequently with deionized water until the total volume reached to the 50 mL mark. The digestion was carried out in triplicates for each sample.

Table 1. Optimization of volume ratio of the reagents for digestion.

S. No.	Volume (mL)	Volume ratio (mL) HNO_3:$HClO_4$:H_2O_2	Temp. (°C)	Time (h)	Observation during volume ratio optimization
1	6	5:0:1	60	2	Yellow solution with too much residue
2	7	6:0:1	60	2	Yellow solution with too much residue
3	8	7:0:1	60	2	Yellow solution with much residue
4	9	8:0:1	60	2	Yellow solution, but relatively lower residue
5	10	8:1:1	60	2	Yellow solution with too much residue
6	11	9:1:1	60	2	Yellow solution with some residue.
7	6	0:5:1	60	2	Dark colored cake like with little liquid
8	7	0:6:1	60	2	Dark and highly turbid solution
9	8	0:7:1	60	2	Dark and highly turbid solution
10	9	0:8:1	60	2	Dark and turbid solution
11	10	0:9:1	60	2	Dark and turbid solution
12	11	0:10:1	60	2	Dark but less turbid solution
13	7	5:2:0	60	2	Light yellow solution with little residue
14	8	6:2:0	60	2	Light yellow solution with very little residue
15	9	7:2:0	60	2	Light yellow solution with very little residue
16	10	8:2:0	60	2	Light yellow solution
17	11	9:2:0	60	2	Light yellow solution
18	12	10:2:0	60	2	Light yellow solution
19	8	5:2:1	60	2	Light yellow solution with little residue
20	9	6:2:1	60	2	Light yellow solution with very little residue
21	10	7:2:1	60	2	Light yellow solution with very little residue
22	11	8:2:1	60	2	Light yellow solution with very little residue
23	12	9:2:1	60	2	Light yellow solution with very little residue
24	13	10:2:1	60	2	Light yellow solution with very little residue
25	**10**	**6:2:2**	**60**	**2**	**A clear light yellow solution with negligible amount of residue on filtration**
26	10	5:2:3	60	2	Clear light yellow solution with some residue
27	10	6:1:3	60	2	Clear light solution with some residue
28	10	7:1:2	60	2	Clear light solution with some residue
29	10	4:4:2	60	2	Clear but brown solution

The bold font shows the optimized volume ratio.

Table 2. Optimization of digestion temperature and time.

S. No.	Total volume (mL)	Optimized volume ratio (mL)	Temp. (^{o}C)	Time (h)	Observation
1	10	6:2:2	60	2	Some yellowish turbid matter
2	10	6:2:2	90	2	The turbid matter decreased
3	**10**	**6:2:2**	**120**	**2**	**Clear and light yellow color solution**
4	10	6:2:2	150	2	Clear light yellow solution
5	10	6:2:2	180	2	Clear light yellow solution
6	10	6:2:2	210	2	Clear light yellow solution
7	10	6:2:2	120	1:00	Yellow solution with some residues
8	10	6:2:2	120	1:15	Yellow solution with some residue
9	10	6:2:2	120	1:30	Yellow solution with some residue
10	10	6:2:2	120	1:45	Yellow solution with some residue
11	10	6:2:2	120	2:00	Yellow solution with some residue
12	10	6:2:2	120	2:15	Yellow solution with some residue
13	10	6:2:2	120	2:30	Light yellow solution with very few residues
14	10	6:2:2	120	2:45	Light yellow solution with very few residues
15	**10**	**6:2:2**	**120**	**3:00**	**Clear light yellow solution**
16	10	6:2:2	120	3:15	Clear light yellow solution

The bold font (trial 3) indicates optimum digestion temperature and the bold font (trial 15) indicates optimum digestion time. Temp. (^{o}C) = temperature.

Digestion of a reagent blank was also performed in parallel with the maize samples keeping all digestion parameters the same. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined.

Determination of metal levels in the samples

Intermediate (10 mg/L) standard solutions were prepared from the stock solutions which were 1000 mg/L in concentration. The intermediate solutions were diluted with deionized water to obtain four working standards for each metal of interest. Then K, Na, Mg, Ca, Fe, Zn, Cu, Pb, Ni and Cr were determined with FAAS equipped with air-acetylene flame system using external calibration curve Mn, Cd and Co concentrations were determined using GFAAS. Three replicate determinations were carried out on each sample. All the thirteen metals were determined by absorption/concentration mode and the instrument readout was recorded for each sample and blank solution. The same analytical procedure was employed for the determination of elements in digested blank solutions.

Instrument calibration

Both the FAAS and GFAAS were calibrated using four series of working standards for each metal of interest. The working standard solutions of each metal were prepared freshly by diluting the intermediate standard solutions. The correlation coefficients of the calibration curves were > 0.997 which confirmed a very good positive correlation between the change in absorbance and the concentration and were linearly fit.

Validation of optimized procedure

The validity of the optimized procedure was assessed by spiking experiments. For this purpose standard solution of 1000 mg/L was used and intermediate standards of 100 mg/L and 10 mg/L were prepared. Thus, spiking was done by classifying the metals in to three groups. In the first group K, Na, Ca and Mg were grouped; Zn, Cu, Fe and Mn in the second and Co, Cr, Cd, Ni and Pb in the third group. The samples were taken from Shendi raw flour for the first group, from Debre Tabor maize bread for the second group and from Finote Selam roasted seed for the third group. This was to consider the forms and sources of samples.

The spiked and non-spiked samples were digested and analyzed in similar conditions using optimized procedure for sample analysis. Then, the percentage recoveries of the analytes were calculated. The results of recovery analysis were within the range 91.2–109%. The percentage recoveries for the samples were between 100±10%, which were within the acceptable range for all metals.

RESULTS AND DISCUSSION

Levels of metals in maize seed samples

Triplicate analysis was made for each metal on triplicate samples and the results are reported as mean ± SD. Among all the studied metals the level of Cd and Ni were below detection limits of the instrument. Results are given in Table 3.

Table 3. Level of metals in mg/kg in maize raw seeds, roasted seeds and bread from Shendi, Finote Selam and Debre Tabor.

Metal	Concentration (mg/kg) (mean ± SD) of metals in samples from								
	Shendi			Finote Selam			Debre Tabor		
	Raw seed	Roasted seed	Bread	Raw seed	Roasted seed	Bread	Raw seed	Roasted seed	Bread
K	1688 ± 49^{a}	1886 ± 22^{d}	1748 ± 50^{g}	1754 ± 46^{a}	1925 ± 83^{d}	1755 ± 166^{g}	1784 ± 151^{a}	1864 ± 111^{d}	1936 ± 100^{h}
Na	163 ± 1^{a}	188 ± 2^{d}	194 ± 1^{g}	126 ± 1^{b}	139 ± 3^{e}	162 ± 2^{h}	150 ± 1^{c}	155 ± 2^{f}	194 ± 2^{i}
Mg	392 ± 4^{a}	402 ± 11^{a}	395 ± 0.7^{a}	397 ± 1^{a}	404 ± 9^{a}	395 ± 6^{a}	388 ± 10^{a}	396 ± 4^{a}	394 ± 0.9^{a}
Ca	102 ± 2^{a}	171 ± 7^{d}	139 ± 5^{g}	72.0 ± 3^{b}	109 ± 1^{e}	123 ± 6^{h}	67.5 ± 1^{c}	124 ± 6^{f}	107 ± 5^{i}
Cr	0.17 ± 0.003^{a}	0.19 ± 0.01^{a}	0.19 ± 0.01^{a}	0.23 ± 0.07^{b}	0.34 ± 0.004^{c}	0.27 ± 0.01^{b}	1.55 ± 0.03^{d}	1.70 ± 0.02^{e}	1.55 ± 0.1^{d}
Mn	2.60 ± 0.1^{a}	2.80 ± 0.05^{d}	2.70 ± 0.13^{g}	1.23 ± 0.2^{b}	1.28 ± 0.2^{e}	0.65 ± 0.1^{h}	3.74 ± 0.2^{c}	4.37 ± 0.2^{f}	2.42 ± 0.07^{i}
Fe	112 ± 3^{a}	101 ± 2^{d}	142 ± 4^{g}	56.8 ± 3^{b}	52.6 ± 0.6^{e}	63.4 ± 2^{h}	19.5 ± 3^{c}	20.0 ± 2.2^{f}	46.0 ± 0.7^{i}
Co	0.48 ± 0.01^{a}	0.70 ± 0.06^{b}	0.44 ± 0.07^{a}	0.43 ± 0.2^{a}	0.58 ± 0.08^{c}	0.48 ± 0.02^{a}	0.46 ± 0.02^{a}	0.66 ± 0.10^{b}	0.69 ± 0.06^{b}
Cu	1.32 ± 0.31^{a}	1.95 ± 0.77^{d}	2.87 ± 0.25^{g}	0.08 ± 0.01^{b}	0.05 ± 0.002^{e}	0.05 ± 0.004^{h}	0.04 ± 0.001^{b}	0.05 ± 0.003^{f}	0.05 ± 0.004^{h}
Zn	61.4 ± 2^{a}	77.6 ± 4^{d}	66.7 ± 5^{g}	72.7 ± 5^{a}	75.1 ± 8^{d}	77.5 ± 3^{h}	69.9 ± 8^{a}	73.3 ± 2^{d}	112 ± 4^{i}
Pb	1.21 ± 0.9^{a}	1.20 ± 1^{a}	2.60 ± 0.8^{b}	1.50 ± 0.5^{a}	2.71 ± 0.4^{b}	2.02 ± 0.6^{c}	1.25 ± 0.03^{a}	2.11 ± 0.5^{c}	2.04 ± 0.5^{c}

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

Levels of metals in maize seeds

There is a variation in the metal concentration of macro-essential metals among the maize samples collected from the three sampling areas. The concentration of K was the highest of all the major metals as shown in Table 3. It was also the highest of all the metals under consideration. It was within the range 1589–2036 mg/kg dry weight, followed by Mg, Na and Ca 303–413, 125–195 and 66.5–178 mg/kg, respectively. That means the concentration profile of macro-essential metals determined in maize seeds was K > Mg > Na > Ca.

Among the sample sites the highest concentration of K was determined in a sample from Debre Tabor (1633–2036 mg/kg dry weight) followed by Finote Selam (1589–2008 mg/kg) and

Shendi (1639–1908 mg/kg). In this study the concentration of K determined by sample sites decreased in the order Debre Tabor > Finote Selam > Shindi. The concentrations of Mg from Shendi and Finote Selam are somewhat the same (388–413 and 389–413 mg/kg), but the value obtained from Debre Tabor is slightly lower than both sample sites (303–400 mg/kg). The amount of Na obtained in the samples from the three sites was as follows: Shendi 161–195 mg/kg, Finote Selam 125–164 mg/kg and Debre Tabor 149–195 mg/kg. This means that the level of Na in the sample from Shendi and Debre Tabor are significantly higher (at $p < 0.05$) than that of Finote Selam. The levels of Ca in the samples were recorded as 100–178 mg/kg, 69.0–129 mg/kg and 66.5–130 mg/kg from Shendi, Finote Selam and Debre Tabor samples, respectively. The sample from Shendi is the highest of the three sites in Ca content. The values of Ca in samples from Finote Selam and Debre Tabor are not significantly different at $p < 0.05$. From Table 3 we can see that Fe is the highest accumulated trace essential metal measured followed by Zn and Mn with concentration ranges 16.5–146 mg/kg, 59.2–116 mg/kg and 0.52–3.98 mg/kg, respectively. It is also clearly seen in Table 3 that the concentration ranges of all the trace metals except Fe and Zn overlap each other among the sample sites. The highest concentration of Fe may be attributed to its higher levels in the soil [26, 27].

Levels of metals in the processed food (roasted seeds and bread)

When we see the levels of macro-essential and micro-essential metals in the raw seed, roasted seed and bread in Table 3 the values were in the order roasted seed > bread > raw seed samples for all metals. Thus the processed foods (roasted seed and the bread) are more nutritious than the raw seeds. This is desirable because people consume the processed food. This is due to the fact that during the roasting of seed and backing of bread the volatile components of the raw seed gets evaporated and minerals get concentrated. The level of toxic metal Pb was also higher in the roasted seed and bread compared to the raw seed. This is also expected but it is undesirable and unavoidable.

Distribution patterns of metals in the samples

The uptake of metals by plants takes place through different and complex biochemical processes. The uptake processes vary based on the ability of the plants to absorb metals from the soil, the availability of the minerals in the soluble and usable forms, the abundance of particular minerals at the particular areas, the degree of contamination of the soil with heavy metals, etc. The differences in the levels of metals in soil arise mainly due to pollution of the biosphere resulting from the rapid industrialization and modern large scale agricultural activities, i.e. use of different types of fertilizers, pesticides and other chemicals.

The use of sewage sludge, pesticides, irrigation with polluted water and fertilizers on agricultural lands highly affect the quality of food products for humans and animals. The distribution and accumulation of metals in maize seeds are the reflections of the mineral composition of the soil and the degree of mineral pollution of the environment in which the maize plant grows. Therefore, the actual metal concentration of maize seeds vary considerably according to the geographic origin, the use of fertilizers with different chemical compositions and other characterizing features such as quality water for irrigation and also the storage conditions of the products.

If we compare the levels of individual metals by sample site the trend can be shown as follows: Shendi > Finote Selam > Debre Tabor in Fe content. Debre Tabor > Shendi > Finote Selam in Mn content. Shendi > Finote Selam ≈ Debre Tabor in Cu content. Debre Tabor > Shendi ≈ Finote Selam in Zn content. Debre Tabor ≈ Shendi ≈ Finote Selam in the level of Co.

The variations in concentrations of Co and Mn are not that much significant and comparable to each other for the three sites. That means the distribution of these metals is invariant in comparison to other metals. The variation of Fe by sample sites was the highest among the micro-essential metals and the variation for Co by sample sites is the least as shown in Table3.

Concentration of non-essential (toxic) metals in maize seeds

According to World Health Organization [28] the dietary exposure to Cd is estimated to be about 1.2 x 10^{-4} to 4.9 x 10^{-4} mg/kg of body weight daily. Intake of dietary Cd should not exceed 0.007 mg/kg of body weight, per week. However, the levels of Cd and Ni in this work were below the instrument detection limits. Using maize seeds from these sites is safe for human consumption and safe from human health problems due to the accumulation of Cd and Ni in maize seeds.

Pb is a major chemical pollutant of the environment, and is highly toxic to man. The values determined for Pb level in this work are presented in Table 3. As we can see from Table 3 the variations among the sites are comparably small, but the amount of Pb determined from Finote Selam is slightly higher than both from Shendi and Debre Tabor. The variation for Pb content in the maize seed by sample site may be attributed to agricultural inputs such as fertilizers herbicides and insecticides containing Pb as an ingredient. Exposure to contamination by dust particles and particulate matters in the air containing Pb during storage and transportation by cultivators could be the other causes for the higher values [29].

The variation on the level of Cr between Shendi and Finote Selam is not significant, but the level in the sample from Debre Tabor is higher. We can compare the differences as Debre Tabor > Finote Selam > Shendi in Cr content.

Comparison of metal levels of the present study with literature values

Comparison of levels of metals obtained in this study has been made with the investigations made in other countries by other investigators. Different researches were being made by different researchers in different countries on maize seed, but in the Ethiopian case no detail studies were made on the levels of major, trace and toxic metal composition in maize seeds.

For the purpose of comparison, the results of this study and results from different literatures are shown in Table 4. As it is seen from the Table 4, the levels of macro-essential metals determined in this work are in good agreement with other studies done in other countries. The concentration of K is in the range 3.24–2662 mg/kg, Na in the range 75.8–3230 mg/kg, Mg in the range 48.94–1154 mg/kg and Ca 0.61–215 mg/kg dry weight. The results of this study (K 1589–2036, Na 125–178, Mg 303–413 and Ca 66.5–178 mg/kg) are all in the ranges.

Table 4. Comparison of macro-essential metals concentration, (mg/kg, dry weight basis) in maize raw seed samples with reported values.

K	Na	Mg	Ca	Country	Reference
1701	536	1154	68.4	Turkey	[16]
2662	75.8	91.5	216	Turkey	[17]
1620	2600	200	20	Brazil	[14]
1950	3230	200	20	USA	[14]
NR	NR	248-321	1.2-10.2	Nigeria	[12]
NR	NR	NR	NR	China	[15]
3.24	3.72	48.9	0.61	Nigeria	[13]
1589-2036	125-195	303-413	66.5-178	Ethiopia	This study

NR = not reported, ND = not detected.

The levels of micro-essential metals in this study and from the literature are shown in Table 5. Also here the values from this study are in good agreement with those from literatures. If we look the values from the literatures [12-17] Fe is in the range 5.9–159 mg/kg, Cr 0.338–2.38 mg/kg, Mn ND to 8.4 mg/kg, Co ND to 0.8 mg/kg and Ni ND to 4.78 mg/kg. The levels of all the metals of this study are in the ranges from the literatures used, i.e. Fe 16.5–146 mg/kg, Cr 0.17–1.72 mg/kg, Mn 0.52–3.98 mg/kg, Co 0.34–0.76 mg/kg and Ni ND are all in the above ranges. In fact the level of Fe in this study is in a higher range (16.5–146 mg/kg), compared to only one literature [17] from Turkey 159 mg/kg. The high value of Fe in this study is most probably resulted from the high concentration of Fe in the soil. Researches in the recent years indicate that Ethiopian soil is high in Fe content, so plants can absorb Fe easily. This could be one reason behind the high value of Fe in Ethiopian maize. [26, 27].

Table 5. Comparison of micro-essential metals concentration, (mg/kg, dry weight basis) in maize raw seed samples with reported values.

Cr	Mn	Fe	Co	Ni	Cu	Zn	Country	Reference
2.38	ND	37.9	NR	0.79	2.85	33.6	Turkey	[16]
1.0	8.4	159	0.8	0.022	3.0	28.4	Turkey	[17]
NR	NR	5.9	NR	NR	0.46	5.0	Brazil	[14]
NR	NR	8.6	NR	NR	0.58	3.9	USA	[14]
NR	NR	28.5-59.5	ND	1.87-4.78	2-10.7	NR	Nigeria	[12]
339*	4.40	28.9	6.58*	123*	2.86	15.0	China	[15]
1.36	ND	8.66	ND	1.04	2.05	6.69	Nigeria	[13]
0.17-1.72	0.52-3.98	16.5-146	0.34-0.76	ND	0.04-2.72	59.2-116	Ethiopia	This study

NR = not reported, ND = not detected, * = concentration µg/kg, the rest are in mg/kg.

Table 6. Comparison of toxic heavy metals concentration, (mg/kg, dry weight basis) in maize raw seed samples with reported values.

Cd	Pb	Country	Reference
NR	NR	Turkey	[16]
ND	1.5	Turkey	[17]
NR	NR	Brazil	[14]
NR	NR	USA	[14]
NR	62.5-150	Nigeria	[12]
32.3*	38.2*	China	[15]
ND	0.32	Nigeria	[13]
ND	0.31-3.41	Ethiopia	This study

NR = not reported, ND = not detected, * = concentration in µg/kg.

The determination of levels of toxic metals was also carried out in this study and Cd was found to be below detection limit of the instrument. The level of Pb was found as 0.31–3.41 mg/kg, when it is compared with the values in the literature (Table 6) it is lower than that mentioned by Olu *et al.* [12] in Nigeria (62.5–150 mg/kg) but higher than most others.

Analysis of variance (ANOVA)

In this study, maize seed samples in three forms were collected from three different areas and the metal levels of each sample was analyzed by AAS. During the processes of sample preparation and analysis a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyte was tested by using one-way ANOVA [30], to examine whether the source for variation was from experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, pH of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, etc. or other variations in cultivation procedures). The source for significant difference between sample means may be due to the differences in mineral contents of soil or pH of soil which predict the extent of mineral absorption by the maize plant. The results in Table 3, shows the significance of the results between samples and within samples at $p < 0.5$.

From Table 3 one can see that there is significant difference at 95% confidence level in mean concentrations of all the metals except K, Mg and Pb in all the three sample forms and the means of Co concentration in raw seed and roasted seed as well as the means of Zn in the form of roasted seed are not significantly different. The source for this significant difference between sample means may be the difference in mineral compositions of the soil or pH of soil which predict the degree of mineral absorption by plants.

Pearson correlation of metals within maize seed samples

In this particular study, to correlate the effect of the concentration of one metal over the other metal, the Pearson correlation coefficients were employed. The relations for the three forms of the maize samples are shown in Table 7A-7C.

Table 7A. Pearson's correlation for maize raw seed samples

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Cu	Zn	Pb
K	1.00										
Na	0.54	1.00									
Mg	0.89	0.86	1.00								
Ca	0.98	0.68	0.96	1.00							
Cr	0.77	0.15	0.36	0.63	1.00						
Mn	0.25	0.69	0.21	0.67	0.82	1.00					
Fe	0.99	0.45	0.84	0.96	0.82	0.35	1.00				
Co	0.58	0.998	0.90	0.73	0.76	0.64	0.64	1.00			
Cu	0.96	0.75	0.98	0.996	0.56	0.25	0.93	0.25	1.00		
Zn	0.70	0.98	0.96	0.82	0.77	0.51	0.63	0.99	0.87	1.00	
Pb	0.91	0.15	0.82	0.61	0.96	0.62	0.95	0.20	0.77	0.35	1.00

Table 7B. Pearson's correlation for maize roasted seed samples.

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Cu	Zn	Pb
K	1.00										
Na	0.45	1.00									
Mg	0.79	0.20	1.00								
Ca	0.38	0.998	0.27	1.00							
Cr	0.71	0.32	0.99	0.38	1.00						
Mn	0.99	0.29	0.88	0.22	0.82	1.00					
Fe	0.25	0.75	0.80	0.80	0.86	0.41	1.00				
Co	0.77	0.92	0.21	0.89	0.09	0.65	0.43	1.00			
Cu	0.16	0.95	0.48	0.97	0.58	0.01	0.92	0.76	1.00		
Zn	0.28	0.98	0.37	0.99	0.48	0.11	0.86	0.83	0.99	1.00	
Pb	0.94	0.13	0.94	0.06	0.90	0.99	0.56	0.51	0.12	0,05	1.00

Table 7C. Pearson's correlation for maize bread samples.

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Cu	Zn	Pb
K	1.00										
Na	0.47	1.00									
Mg	0.99	0.50	1.00								
Ca	0.88	0.010	0.87	1.00							
Cr	1.00	0.45	0.99	0.89	1.00						
Mn	0.36	0.99	0.39	0.13	0.34	1.00					
Fe	0.67	0.34	0.64	0.94	0.68	0.46	1.00				
Co	0.99	0.36	0.99	0.93	0.99	0.24	0.75	1.00			
Cu	0.53	0.50	0.50	0.87	0.54	0.60	0.98	0.63	1.00		
Zn	0.50	0.50	1.00	0.86	0.99	0.39	0.64	0.99	0.50	1.00	
Pb	0.99	0.53	0.47	0.85	0.52	0.63	0.98	0.60	1.00	0.47	1.00

The values of Pearson correlation coefficient in Table 7A-7C revealed that there is weak and/or moderate positive correlation between metals with each other except for some metals. The weak correlation indicating that the presence or absence of one metal affects the other metal in a lesser extent. As we can see from the correlation tables there is a very high positive correlation of K with Fe in the case of raw seeds, with Mn in the case of roasted seeds and with Cr, Pb and Mg in the case of bread, Na with Zn and Co both in flour and bread samples. Ca with Fe, Cr with Pb, Mn with Pb, Fe with Cu and Pb as well as Cu with Pb and Zn can be mentioned as strong correlations can be seen from the table. These strong correlations may arise from common anthropogenic or natural sources as well as from similarity in chemical properties. Mn show week to medium correlation with most of the metals except the strong relations with K, Na and Pb.

Daily intake of metals from maize seed

Daily intake of metals from maize seed food has been calculated based on the assumption that an average adult person consumes 200 g dry maize food per day on the average. The amounts of mineral intake by the person from the different forms of maize seed food are given in Table 8.

Table 8. Comparison of daily intake of metals from maize seed food with recommended daily intake and tolerable upper limit of daily intake of metals.

Metal	Concentration in maize (mg/kg)	Amount of metal per 200 g maize consumed	Recommended daily intake (RDI) [28, 31]	Tolerable upper limit [28, 31]
Ca	66.5–178	13.3–35.6	1000–1200 mg	2500 mg/day
Mg	303–413	60.6–82.6	320–420 mg	750 mg/day
K	1589–2036	318–407	4700 mg	ND
Na	125– 195	25–39	1500 mg	2300 mg/day
Cr	0.17–1.72	0.03–0.25	25–35 µg	120 µg/day
Cu	0.04–2.72	0.01–0.54	0.9–2 mg	10 mg/day
Fe	16.5–146	3.3–29.2	10–15 mg	45 mg/day
Mn	0.52–3.98	0.104–0.796	1.8–2.3 mg	11 mg/day
Ni	ND	None	70–170 µg/kg	1 mg/day
Zn	59.2–116	11.8–23.2	10–15mg	40 mg/day
Cd	ND	None	ND	7 µg/kg body wt/week
Pb	0.31–3.41	0.06–0.68	0.02–3µg/kg body wt	25 µg/kg body wt/day
Co	0.34–0.76	0.07–0.15	5–40 µg/day	0.25 mg/day

ND = not determined (not established).

The amount of all major metals (Ca, Mg, K and Na) that a person can get is lower than the daily recommended values; this indicates that maize alone cannot be a good source of the major metals needed for the daily requirement for the major metals. Therefore the person must get supplementary Ca, Mg, K and Na from other sources. The amount of Mn and Cu that the man can get is also below the required amount. Hence supplementary diet is needed for these metals too. The amount of iron is very sufficient, for the sample from Shendi but below the required limit for those from the other two sites, so additional source of Fe is needed for those people in the two sites. The amount of Zn that a man can get from maize is in the range for those from Shendi and Finote Selam, the amount from Debre Tabor is higher than the RDI, but it is still below the maximum limit. The values for Co, Cr and Pb are all above the allowable limits. Maize in these areas has much higher content of these metals. A man must not consume foods from maize regularly. Since the levels of Cd and Ni in samples from the three sites are below the detection limit, it is possible to conclude that, a person is free from the risks of Cd and Ni as a result of consuming maize.

CONCLUSION

An efficient digestion procedure for the determination of metals in the raw maize seed and its processed food (roasted seed and bread) samples was developed and validated through spiking method and a good percentage recovery was obtained (100±10%) for all the metals of interest. The levels of metals in maize seed determined in this study was in the order: K (1589–2036 mg/kg) > Mg (303–413 mg/kg) > Na (125–195 mg/kg) > Ca (66.5–178 mg/kg) > Fe (16.5–146 mg/kg) > Zn (59.2–116 mg/kg) > Mn (0.52–3.98 mg/kg) > Pb (0.31–3.41 mg/kg) > Cu (0.04–2.72 mg/kg) > Cr (0.17–1.72 mg/kg) > Co (0.34–0.76 mg/kg).

From the nutritional point of view processed maize foods are better sources of minerals than raw seed. The non-essential/toxic heavy metal, Cd and the other trace metal, Ni were found below the detection limits of the instrument. It was concluded that maize seeds from the selected sites accumulated relatively larger amounts of K and Fe but low levels of Cu (0.04–0.08 mg/kg.

The ANOVA results at 95% confidence level suggests that there were significant difference in the mean concentration of all metals except for K, Mg and Pb among the sampling areas. These differences could be attributed to the difference in mineral contents of soil or pH of soil which predict the extent of mineral absorption by maize plants. Generally, the level of essential metals in maize seed determined in this study could be put in the following order K > Mg > Na > Ca > Fe > Zn. Mn, Co and Cu are not significantly in different ranges. The non-essential heavy metal, Pb and Cr are also not significantly in different ranges. The levels of Cd and Ni were below the detection limits of the instrument. Statistical analysis using one way ANOVA indicates that there is significant difference in mean concentration of metals under investigation except K, Mg and Pb. This may be attributed to differences in soil composition, use of different fertilizers, pesticides, and may also be resulted from random errors in the experimental processes. The concentrations of Pb determined in this work were relatively lower than most of the values in the literatures mentioned, but it is higher than the recommended values. This higher value may be due to the anthropogenic sources or from the ingredients of agricultural inputs, like fertilizers, insecticides and herbicides used. These results inform the consumer of the safety of maize seeds and its processed foods grown in Shedi, Finote Selam and Debre Tabor, Ethiopia.

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