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MINERAL CONTENTS OF BARLEY GRAINS AND ITS PROCESSED FOODS (KOLO, PORRIDGE, BREAD AND INJERA) CONSUMED IN ETHIOPIA

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ABSTRACT. Barley (*Hordeum vulgare* L.) is one of the most widely cultivated stable food crops in the world. Barley grain samples were collected from four selected areas (Bahir Dar, Bure, Finote Selam and Debre Markos) of Ethiopia and four types of processed food (kolo, porridge, bread and injera) were prepared from it. The levels of essential and non-essential metals in barley grains and its processed food were determined by microwave plasma-atomic emission spectrometry after wet digestion with a mixture of HNO_3 and $HClO_4$ (5:1, v/v). The concentration (mg/kg dry weight) in the barley grains were in the ranges K (5482-6516), Mg (546-643), Ca (445-684), Mn (7.31-9.80), Fe (127-439), Cu (0.88-1.86), Zn (42.8-56.8), Pb (0.39-2.73), Cd (3.01-4.66). The concentrations of all the metals in the four types of processed barley foods showed variation among each other. The results indicate that Ethiopian barley grains and its processed foods are good source of essential metals.

KEY WORDS: Barley, *Hordeum vulgare* L., Processed foods, Macro-minerals, Micro-minerals, Toxic metals

INTRODUCTION

Cereals are common food crops of the world. They provide food calories and proteins to human. They are staple foods for most of the population. Barley (*Hordeum vulgare* L.) is a cereal crop. It belongs to the tribe Triticeae of family Poaceae. It is an important cereal crop and cultivated over broad environmental conditions in the world [1-3]. It ranks fourth in the world in production after wheat, maize and rice [4].

Barley is used commercially for animal feed, to produce malt, and for human food applications as it is rich in protein, carbohydrates, dietary fibers, minerals, vitamins and antioxidants [5, 6]. Barley is also an important crop in Ethiopia [3]. It is ranked fifth among the cereals on the basis of area of cultivation while third on the basis of production per unit area in Ethiopia. It covers 7.56% of the land under grain crop cultivation with a yield of 1.96 tones/ha [7].

Ethiopia is second largest producer of barley in Africa next to Morocco, accounting for about 26% of the total barley production in the continent [8, 9]. It serves as a component of various foods. It is used in soups and stews, and the grain is used locally in bread, biscuits, and the traditional beremeal bannock. Barley is used commercially for animal feed, to manufacture malt, which is primarily used in beer production, and for human food applications [10, 11]. It is used for the preparation of Ethiopian fermented bread injera, porridge, roasted snack, and in homemade beer. Barley provides basic necessities of life (food, feed, and beverages) for many people in the Ethiopian highlands [2]. Barley is used in the preparation of different recipes in Ethiopia. It is deep rooted in the culture of people's diets. It has also been used for the preparation of various types of traditional foods such as kolo (roasted grains), kita (fried bread), dabo (baked bread), beso (snack), genfo (porridge), chuko (barley conserved with butter), tihlo (dough balls), shorba (soup), kinche (cooked broken grains), and injera (pan cake bread) in Ethiopia [12, 13].

Barley grain consists of about 65-68% starch, 10-17% protein, 2-3% free lipids, 4-9% β-glucans and 1.5-2.5% minerals. Total dietary fiber ranges from 11-34% containing soluble dietary

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fiber within 3-20% [14]. Barley grain also contains important minerals such as zinc (up to 50 mg/kg), iron (up to 60 mg/kg) and soluble fibers. It also contains higher amounts of vitamins A and E than the other cereals [2].

Chemical composition and physical characteristics of cereal grains used in human and livestock feeding have been reported in many studies [15]. Variations in the chemical composition and physical characteristics of cereal grains results from changes in the environmental factors, such as rainfall, temperature, soil conditions, fertilization and genetic factors [16, 17]. The characterization of variations in nutritional values of cereal grains that result from environmental factors and soil properties may help for improving the quality of cereal grains [17].

Minerals are considered to be essential in human nutrition and that they are important constituents of bones, teeth, tissues, muscles, blood, and nerve cells. Generally, the minerals help in the maintenance of acid-base balance, the response of nerves to physiological stimulation, blood clotting, structural, physiological, catalytic, and regulatory [18]. Minerals may be broadly classified as macro (major), micro (minor), or trace and toxic [19]. The macro-minerals required in amounts of 100 milligrams or more per day include calcium, magnesium, phosphorus, sodium, sulfur, and chloride. Micro-elements or trace elements are present at low levels in the body or required in smaller amounts in the diet, including iron, copper, cobalt, dine, zinc, manganese, molybdenum, fluoride, chromium and selenium. The micro-minerals are required in amounts less than 100 milligrams per day [19]. Toxic elements such as mercury, arsenic, cadmium, and lead, can diminish mental and central nervous system function; cause damage to blood composition as well as the kidneys, lungs, and liver; and reduce energy levels [20].

Some studies have been conducted on the levels of minerals in the raw and processed foods in Ethiopia. Atlabachew and Chandravanshi [21] determined the metal contents in commercially available enset food products (kocho and bulla). Debebe *et al.* [22] studied the metal contents in enset corm. Aregahegn *et al.* [23] reported the metal contents in tubers and flour of *Dioscorea abyssinica*. Akalu and Chandravanshi [24] determined the selected metals in raw and processed *Lupinus albus* L. Ayele *et al.* [25] studied the mineral content and antinutritional factors of yam and taro during cooking. Abebe and Chandravanshi [26] and Abebe *et al.* [27] reported the metal contents of raw maize and its processed food. Tegegne *et al.* [28] studied the mineral contents of raw and cooked rice. However, no systematic study has been done of barley grain and its processed food in Ethiopia.

Therefore, it is worthwhile to assess the nutritional value of barley and its food products in different geographic locations having different environmental conditions. Hence, this study is aimed to investigate the mineral compositions of barely grain and its processed foods. Previously published studies have reported higher contents of calcium, iron and zinc in barely than other common cereals [12, 13, 29]. However, mineral contents in the barely flours is variable and controversial in the literature [12, 13, 29]. The data on mineral contents in barely grains and its processed foods are scarce and limited. Therefore, the aim of present investigation is to determine the amount of minerals in barely flours and barely processed foods by microwave plasma-atomic emission spectrometry.

EXPERIMENTAL

Apparatus and equipment

Ceramic mortar and pestle was used to ground barley samples. Electronic series balance (OPTECH, A205EC, Italy) with precision of ±0.0001 g was used for weighing samples. A Kjeldahl (UK) digestion block was used for the digestion of barley samples. Microwave plasma-atomic emission spectrometer (Agilent 4200, MP-AES, USA) was used for quantification of metals.

Chemicals

Analytical grade reagents and chemicals were used in the study. A 69.5% HNO_3 (Scharlau Chemie S.A. European Union, Spain) and 70% $HClO_4$ (BDH Laboratory Supplies AnalaR®, Poole, England) were used for digestion of barely samples. Stock standard solutions containing 1000 mg/L of the metals K, Mg, Ca, Mn, Fe, 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 throughout the study.

Description of the study area

The study was conducted in four locations (Bahir Dar, Bure, Finote Selam and Debre Markos). Bahir Dar is situated on the southern shore of Lake Tana, the source of Blue Nile River. It is about 549 km northwest of Addis Ababa at an altitude of 1801 masl, having latitude of 11038"N and longitude of 37010 "E. It is the most heavily populated belt of the Ethiopian plateau. Its area is about 16,000 hectares [30]. Bure is located about 150 km South West of Bahir Dar with longitude and latitude of 10°42'N and 37°4'E. Its annual rainfall ranges from 1386 to 1757 mm [31]. Finote Selam is located in the West Gojjam Zone of the Amhara Region. It has longitude and latitude of 10°42'N, 37°16'E, 10.7°N, 37.267°E, with an elevation of 1917 masl [32]. Debre Markos is located in the north west of Addis Ababa at a distance of 298 km. Its area is 6,160 ha. Its average annual temperature is 18.5 ºC; mean annual rainfall is 1,380 mm [33]. The geographical descriptions of sample collection sites are summarized in Table 1.

Table 1. Geographical descriptions of sample collection sites.

S. No.	Sample site	Approximate geographical locations			
		Latitude	Longitude	Altitude above sea level (m)	Distance from Addis Ababa (km)
1	Debre Markos	10º20′N	37º43′E	2446	298
2	Fenote-Selam	10º42′N	37º16′E	1957	388
3	Bure	10º42′N	37º4′E	2091	400
4	Bahir Dar	11º36′N	37º23′E	1800	549

Sample collection

Barley samples were collected from Bahir Dar, Bure, Fenote-Selam and Debre Markos. About 0.5 kg of samples was collected from three farmers in the open markets separately from each sampling site. The samples from the three farmers from each site were mixed to get about 1.5 kg composite sample from each site, packed in the polyethylene plastic bags and were brought to Addis Ababa for analysis.

Description of processed foods from barely

In Ethiopia, barley is a dependable source of food in the highlands areas. Its grain is used for the preparation of different foodstuffs, such as kolo, porridge, bread and injera, and local drinks, such as tella, borde and beer [9]. This study is concerned on four types of barley foods such as kolo, porridge, bread and injera.

Kolo is a dehulled, well-roasted barley whole grain that is eaten as a snack, alone or mixed with roasted seeds such as ground nut, chick pea, sunflower or safflower seeds. Kolo is commonly eaten both at home and at various social gatherings, such as after funerals ceremonies, during many official meetings or during traveling. Kolo is also eaten by people having gastritis problems [34].

Porridge

Porridge is widely eaten breakfast foods in Ethiopia. It is most commonly consumed during a special celebration such as birthdays and weddings. Traditionally, in many parts of Ethiopia, there is a common practice to prepare barley porridge for an expectant mother. A postnatal mother eats porridge with spiced butter for breakfast and her guests are also served porridge. Traditionally porridge is also considered as a supplementary food for children aged between 6 months and 24 months to make the baby grow faster and stay healthy [12].

Bread

Bread is the most widely eaten food. It has desirability to all population rich and poor, rural and urban. It is a good source of nutrients, such as macronutrients (carbohydrates, protein and fat) and micronutrients (minerals and vitamins) that are all essential for human health. The widespread consumption of bread in the world has necessitated the study of the composition of the bread to improve its nutritive value [35].

Injera

Injera is a fermented, pancake-like soft, circular flat bread with small bubbly structures or eyes (honey-comb-like holes) on its top surface, which are produced due to the production and escape of CO_2 during fermentation and baking, respectively. It is prepared from various cereals depending on availability such as teff, barley, sorghum, maize, wheat and rice or a combination of some of these cereals [3].

Sample preparation for metal determination

Samples were washed with tap water to remove the adsorbed soil and other particulate matters and the barley grains were placed in the hot water for 10 min. The clean grains were crushed in the traditional mortar to remove kernel and dried in the air. The dried grains were separated from the kernel by winnowing and rinsed with deionized water. The samples were exposed to sunlight in open air for drying.

A 200 g portion of the dried barley grains were ground using electronic blending device and stored in sample bottles. The flour was used to prepare porridge, bread and Injera.

A 200 g barley grain samples were roasted using metal pans, cooled and ground using the electronic blending device. The roasted grain is called “kolo” in Ethiopia. The powdered kolo sample was stored sample bottles.

The porridge was prepared according to Ethiopian traditional procedure. A 200 g barley flour was added to the 100 mL of boiling distilled water and homogenized with wooden spoon for 5-10 min. The prepared porridge was put in to oven at 200 ºC for 3 h to complete dryness. The dried porridge sample was ground to powder and stored sample bottle.

The bread was prepared according to the traditional procedure used in Ethiopia. A 200 g barley flour and 0.5 g starter (yeast) was mixed well with 200 mL distilled water and after 1 h the dough was fermented. The metal pan was heated and the dough was placed on the pan. The pan was covered with lid, and after 10 min the bread was inverted upside down for uniform heating. The bread was taken off from the pan and allowed to cool. It was cut in to pieces and exposed to oven at 150 ºC for 3 h to complete dryness until a constant weight was obtained. The dried bread sample was crushed in to powder form and stored in the sample bottle.

Injera was made by mixing 200 g barley flour with 200 mL distilled water to make a dough, and triggering a fermentation process by inoculating the dough with yeast, a starter culture, left over from a previous fermentation. The fermentation lasted 3 days, then 200 mL boiled distilled water was added after which the dough was thinned into a batter before baking on an open platter. A clay plate was heated and the dough was placed on the plate. The plate was covered with lid, and after 5 min the injera was taken off from the plate and allowed to cool. The injera was cut in to pieces and exposed to oven at 150 °C for 3 h to complete dryness. The dried injera sample was crushed in to powder form and stored in the sample bottle.

It should be noted that traditionally the heated metal pan is used for preparing bread and kolo. Therefore, the heated metal pan was used for preparing bread and kolo in the present study. Normally contamination occurs when liquid sample is in contact with the metal pan. However, the contamination will be negligible when a solid sample is in contact with the metal. Therefore, we expect negligible contamination of metals from the metal pan during preparation of bread and kolo.

Optimization of digestion procedure

Wet acid digestion is one of the methods commonly used for samples of organic matrix. It is based on changing digestion parameters like temperature, volume ratio of reagent and time. Kjeldahl apparatus is one of the wet acid digestion apparatus by which organic component are assumed to be decomposed in the form of different gases leaving metallic elements in the residue. Several trials for the optimization of digestion procedure for sample preparation were made as shown in Table 2.

Table 2. Optimization of reagent volume, temperature and time for digestion of 0.5 g of barley sample.

Sample No.	Total volume (mL)	Optimized volume ratio (mL) (HNO_3:$HClO_4$)	Temp. (°C)	Time (h)	Observation
1	6	3:3	240	4:00	Colorless solution
2	6	4:2	240	4:00	Colorless solution
3	**6**	**5:1**	**240**	**4:00**	**Clear colorless solution**
4	8	5:3	240	4:00	Colorless solution
5	5	3:2	240	4:00	Colorless solution
6	5	4:1	240	4:00	Colorless solution
7	7	5:2	240	4:00	Light yellow solution
8	9	6:3	240	4:00	Colorless solution
1	6	5:1	150	4:00	Light yellow solution
2	6	5:1	180	4:00	Light yellow solution
3	6	5:1	210	4:00	Colorless solution
4	6	5:1	240	4:00	Colorless solution
5	**6**	**5:1**	**270**	**4:00**	**Clear colorless solution**
6	6	5:1	300	4:00	Light yellow solution
1	6	5:1	270	1:30	Light yellow solution
2	6	5:1	270	2:00	Light yellow solution
3	6	5:1	270	2:30	Colorless solution
4	6	5:1	270	3:00	Colorless solution
5	6	5:1	270	3:30	Colorless solution
6	**6**	**5:1**	**270**	**4:00**	**Clear colorless solution**

The bold font indicates the optimum condition.

Digestion of samples

A 0.5 g barley grain flour from the prepared sample were transferred to a 250 mL round bottom and 6 mL (5 mL 69.5% HNO_3 and 1 mL 70% $HClO_4$) was added to it. The mixture was digested on a Kjeldhal digestion block for the optimized period of 4 h at the optimized temperature 270 °C. After 4 h of the digestion time the digested mixture was cooled to room temperature for about

30 min without removing the condenser. After removing the condenser about 10 mL distilled water was added to the solution by rinsing the neck of the round bottom flask and the tip of the condenser which was in contact with the flask to dissolve the precipitate formed on cooling and to reduce dissolution of filter paper by digest residue during filtration using Whatman filter paper in to 50 mL volumetric flask. The solution was diluted to 50 mL with deionized water.

A 0.5 g of the four types of processed foods from barley were also digested separately in the same manner as the barley grains.

Instrument calibration

MP-AES was calibrated using four series of working standards for each metal of inters. The working standard solution of each metal was prepared by diluting the standard solutions. Concentrations of the working standards, correlation coefficients of the calibration curves for each metal are listed in Table 3. From the correlation coefficients in Table 3 for each metal it is possible to conclude that the change in the emission intensity with concentration is in good positive correlation and are linearly fit.

Method detection and quantification limits

The method detection limit (MDL) and method quantification limit (MQL) were determined as three and ten times the standard deviation of blank solutions, respectively [36]. The results are given in Table 4 which clearly indicated that the studied metals can be determined at trace levels in the barley grain and its processed foods.

Table 3. The wavelength, correlation coefficient and equation of the calibration curves for determination of metals using MP-AES.

Metals	Wavelength (nm)	Concentration of working standards (mg/L)	Correlation coefficient (R^2)	Equation for calibration curves
K	766.491	0, 5, 10, 15, 20	$R^2 = 0.9999$	I = 25338C–4.9378
Mg	285.213	0, 5, 10, 15, 20	$R^2 = 0.9999$	I = 105108C+14221
Ca	422.673	0, 5, 10, 15,20	$R^2 = 0.9984$	I = 85968C+30355
Mn	403.076	0, 5, 10, 15, 20	$R^2 = 0.9999$	I = 27657C–0.0332
Fe	371.993	0, 5, 10, 15, 20	$R^2 = 0.9999$	I = 5056.6C–0.5136
Cu	324.754	0, 5, 10, 15, 20	$R^2 = 0.9999$	I = 106700C+2.83
Zn	213.857	0, 5, 10, 15, 20	$R^2 = 0.9999$	I = 5270.3C–0.0354
Pb	405.781	0, 5, 10, 15, 20	$R^2 = 0.9999$	I = 2926.7C–0.0692
Cd	228.802	0, 5, 10, 15, 20	$R^2 = 0.9998$	I = 7851.3C+919.77

I = emission intensity, C = concentration.

Table 4. Instrument method detection limits for the analysis of barley sample by MP-AES.

Metals	K	Mg	Ca	Mn	Fe	Cu	Zn	Pb	Cd
MDL(mg/g)	0.1	0.6	0.5	0.01	0.05	0.03	0.06	0.006	0.006
MQL(mg/g)	0.3	1.9	1.8	0.02	0.1	0.05	0.2	0.02	0.02

Validation of optimized procedure

Spiking experiments were used to validate the optimized procedure. The samples were spiked with known concentration of each metal and digested and analyzed in similar conditions using optimized procedure for sample analysis. The percentage recoveries were obtained within the range 93.4-107% which is within the acceptable range for all metals.

Statistical analysis

The statistical software (SPSS Version 22) was used for the analysis of variance (ANOVA) and Pearson correlation. The graphical expression was done using Microsoft Excel 7 in addition to data analysis.

RESULTS AND DISCUSSION

Levels of metals

Each sample was analyzed in triplicate. The mean values were determined from the results of triplicate analysis of each sample for each metal and the results are reported in terms of mean values ± SD (Table 5 and 6).

Table 5. Metals concentration in barley grain samples from four different areas of Ethiopia.

Metal	Metal concentration (mean±SD)			
	Bahir Dar	Bure	Fenote Selam	Debre Markos
K	6516 ± 0.5	6156 ± 0.8	6110 ± 0.4	5482 ± 0.3
Mg	643 ± 0.4	554 ± 0.3	546 ± 0.3	551 ± 0.8
Ca	357 ± 0.86	363 ± 0.4	445 ± 0.86	684 ± 0.9
Mn	7.63 ± 0.3	7.31 ± 0.3	9.42 ± 0.6	9.8 ± 0.9
Fe	213 ± 0.6	127 ± 0.6	439 ± 0.3	128 ± 0.6
Cu	1.86 ± 0.1	0.88 ± 0.1	1.15 ± 0.1	0.97 ± 0.1
Zn	56.8 ± 0.3	42.8 ± 0.3	46.7 ± 0.3	45.3 ± 0.9
Pb	2.73 ± 0.2	0.39 ± 0.05	0.98 ± 0.1	0.87 ± 0.1
Cd	4.66 ± 0.1	3.75 ± 0.2	3.51 ± 0.1	3.01 ± 0.1

Distribution of metals in barley grain sample

The plants uptake metals by different and complex biochemical processes. The accumulation of metals depends on the ability of particular plant to absorb metals from the soil and the availability of the minerals in the soluble forms in the particular areas. The variation in the level of metals in soil depends on the degree of pollution of the biosphere from the rapid industrialization and modern large scale agricultural activities [34-36]. The use of sewage sludge, pesticides, herbicides and fertilizers on agricultural lands highly affect the quality of food products for humans and animals. The distribution and accumulation of metals in barely grain are the reflections of the mineral composition of the soil and the degree of mineral pollution of the environment in which the barely plant grows. The metal concentrations of barley grain vary considerably at different locations due to differences in composition of chemicals and fertilizers and also due to variation in the soil composition and climatic conditions [34-36].

Levels macro essential metals in barley grains

There was a wide variation in the level of macro-essential metals among the samples from the four different sites (Table 5). K content was at the highest level among all the major metals. Its content was also at the highest level among all the metals determined in this study. It was within the range 5482-6516 mg/kg, followed by Ca (445-684) and Mg (546-643) mg/kg. Thus order of macro-essential metals in barely grain was K > Ca > Mg. Among the sample sites the highest concentration (mg/kg) of K was in the sample from Bahir Dar (6516 mg/kg) followed by Bure

(6156 mg/kg), Fenote Selam (6110 mg/kg) and Debre Markos (5482 mg/kg). The order of K concentration by sample sites was Bahir Dar > Bure > Finote Selam > Debre Markos. The concentration (mg/kg) of Mg from Bahir Dar was (643 mg/kg), Bure (554 mg/kg), Debre Markos (551 mg/kg) and Finote Selam (546 mg/kg). The levels of Ca in the samples were found as 684, 445, 363 and 357 mg/kg from Debre Markos, Fenote Selam, Bure and Bahir Dar samples, respectively. Debre Markos sample was higher in Ca content than the samples from other three sites.

The major metals in barley grains showed variations among the sample sites. This might be due to the differences in the availability of the minerals in the soluble and usable forms, differences in the natural occurrence of these minerals in the areas, and differences in the degree of contamination of the soil by these metals. The use of different fertilizers, soil acidity and water for irrigation could also be the causes for the differences [34-36].

Table 6. Level of metals in mg/kg in barley kolo, porridge, bread and injera from Bahir Dar, Bure, Fenote Selam, Debro Markos.

Metal	Concentration (mg/kg) (mean ± SD) of metals in samples from															
	Bahier Dar				Bure				Fenote Selam				Debre Markos			
	K	P	B	I	K	P	B	I	K	P	B	I	K	P	B	I
K	4351 ± 0.3	4735 ± 0.5	4686 ± 0.9	4776 ± 0.4	3839 ± 0.5	4022 ± 0.4	4241 ± 0.9	428 ± 0.06	4129 ± 0.35	4148 ± 0.4	4351 ± 0.3	4158 ± 0.4	4609 ± 0.7	4592 ± 0.35	4762 ± 0.6	4525 ± 0.5
Mg	640 ± 0.6	767 ± 0.8	744 ± 0.3	763 ± 0.9	646 ± 0.6	655 ± 0.6	649 ± 0.4	681 ± 0.6	602 ± 0.3	659 ± 0.5	614 ± 0.6	645 ± 0.8	597 ± 0.3	653 ± 0.8	674 ± 0.8	684 ± 0.3
Ca	395 ± 0.4	518 ± 0.6	430 ± 1.3	891 ± 0.86	598 ± 0.3	658 ± 0.56	622 ± 1.3	727 ± 1.4	437 ± 0.8	397 ± 0.9	547 ± 1	518 ± 1.8	409 ± 0.8	422 ± 0.9	626 ± 0.86	610 ± 0.6
Mn	7.34 ± 0.3	8.91 ± 0.7	9.29 ± 0.1	9.74 ± 0.1	7.52 ± 0.5	9.00 ± 0.4	8.75 ± 0.04	10.5 ± 0.2	7.14 ± 0.3	8.4 ± 0.1	8.5 ± 0.2	8.5 ± 0.1	8.0 ± 0.2	8.3 ± 0.2	9.5 ± 0.1	11.7 ± 0.6
Fe	128 ± 0.2	315 ± 0.5	336 ± 0.4	387 ± 0.3	130 ± 0.9	145 ± 0.5	193 ± 0.6	206 ± 0.6	131 ± 0.5	241 ± 0.5	204 ± 0.5	360 ± 0.6	231 ± 0.6	306 ± 0.5	266 ± 0.6	348 ± 0.9
Cu	1.58 ± 0.08	4.78 ± 0.09	6.74 ± 0.11	2.86 ± 0.06	1.61 ± 0.04	2.47 ± 0.03	2.45 ± 0.03	2.6 ± 0.05	2.21 ± 0.04	2.7 ± 0.03	2.4 ± 0.1	6.27 ± 0.06	4.10 ± 0.08	5.25 ± 0.05	4.51 ± 0.12	5.82 ± 0.09
Zn	50 ± 0.5	65 ± 0.9	66 ± 0.5	60 ± 0.5	40 ± 0.6	46 ± 0.7	43 ± 0.1	48 ± 0.7	45 ± 0.1	39 ± 0.8	42 ± 0.7	44 ± 0.5	40 ± 0.5	42 ± 0.5	42 ± 0.6	44 ± 0.1
Pb	1.71 ± 0.75	2.16 ± 0.56	5.32 ± 0.33	3.13 ± 0.4	1.59 ± 0.23	1.88 ± 0.12	2.46 ± 0.16	2.33 ± 0.4	2.08 ± 0.37	1.7 ± 0.11	3.02 ± 0.09	2.87 ± 0.13	2.2 ± 0.08	2.43 ± 0.08	2.63 ± 0.2	2.69 ± 0.6
Cd	3.31 ± 0.07	3.81 ± 0.1	4.08 ± 0.06	3.21 ± 0.06	2.93 ± 0.06	2.79 ± 0.05	3.76 ± 0.09	4.25 ± 0.1	2.98 ± 0.08	3.09 ± 0.07	2.76 ± 0.07	2.48 ± 0.02	3.15 ± 0.04	2.46 ± 0.09	1.6 ± 0.04	1.86 ± 0.07

K = Kolo, P = Porridge, B = Bread, I = Injera.

Levels of essential trace metals in barely grains

It can be clearly seen from Tables 5 that Fe is the highest accumulated trace essential metal. This may be due to the nature of the soil produced either from fertilizer or animal and plant decomposition followed by Zn, Mn and Cu with concentration ranges 42.8-56.8, 7.31-9.8 and 0.88-2.73 mg/kg, respectively. The overall concentration of trace essential metals in barely grain was in the order Fe > Zn > Mn > Cu. The metals in the barley grains were in the same order in all the four sites. The lower concentration of trace minerals in the barley grain may be due to soil characteristics. There was a higher degree of variation in the level of Fe by sample sites. It was also at the highest level among the micro-essential metals. Cu exhibited small variation among the four sample sites as shown in Table 5.

Levels of toxic metals in barley grains

The levels of Pb and Cd found in the present work are presented in Table 5. The amount of Pb and Cd determined in the barley grain from Bahir Dar was higher than from Bure, Fenote Selam and Debre Markos. The amount of Pb and Cd in the barley grain from Bahir Dar may be due to use of different fertilizers and pesticides containing Pb and Cd as an ingredient. Exposure to contamination during storage and transportation by cultivators could be the other causes for the higher values. The dietary exposure to Cd and Pb were estimated to be about 0.8 µg/kg of body weight daily and 0.02-3 µg/kg of body weight daily, respectively by the World Health Organization [4]. Accordingly the dietary intake of Cd and Pb should be less than 0.007 mg/kg of body weight, per week and 25 µg/kg of body weight daily, respectively [4].

Concentration of metals in barley food samples

The levels of metals in barley foods from the four sample sites were determined and are given in Table 6. There is a wide variation in the levels of metals in the four types of barley processed foods. Among the four types of barley foods kolo contains lowest amounts of all the metals. This is expected because during the preparation of kolo the barley grains are roasted which results in opening of the grains and subsequent loss of metals. In contrast to kolo, barley injera contains highest amounts of almost all the metals. This is because injera is baked at relatively lower temperature than the roasting of barley grains during kolo preparation. Barley porridge contains slightly lower amounts of metals than the injera but relatively higher levels of metals than the kolo and bread. Barley bread contains relatively higher levels of metals than the kolo but lower levels of metals than the injera and porridge.

There is no general trend in the levels of toxic metal Cd and Pb in the four types of barley processed foods. The highest amount of Cd (4.66 mg/kg) was obtained in the Bahir Dar injera while the lowest amount of Cd (1.6 mg/kg) was found in the Debre Markos bread. The highest amount of Pb (5.32 mg/kg) was observed in the Bahir Dar bread and lowest amount of Pb (1.50 mg/kg) was found in the Bure kolo sample. The variation in the levels of Cd and Pb in the four types of barley processed food from four different areas may be due to use of different types and amounts of fertilizers and pesticides and differences in the nature and sources of irrigation water.

The variation in the levels of metals in the barley grain and its processed food depends up on the nature of individual metal, differences in the food processing, soil properties, environmental conditions and use of different fertilizers and pesticides. The variation in the levels of metal in the four types of barley foods may be also due to differences in the nature and sources of irrigation water and overall in the nature of soil and climatic conditions in the four different areas of barley cultivation.

It should be noted that there is wide variation in the mineral contents of barley grains collected from the four different sampling sites. There is also a wide differences in the preparation of four types of barley foods. The variation in the mineral contents of the barley grains from the four sampling sites is due to variations in the geographical locations and environmental conditions of the four sampling sites. While the variations in the mineral contents of the barley grains and the processed foods from the four sampling sites are due to variations in the geographical locations and environmental conditions of the four sampling sites as well as the variations in the processing of four types of barley foods. These two factors resulted in the random variations in mineral contents in the barley grains and its processed foods.

Comparison of metal levels of present study with literature values

Several studies have been done on barley grain by different investigators in different countries. However, there is no detailed study conducted on the levels of metal contents in barley grains cultivated in Ethiopia. Therefore, the results of present study have been compared with the results reported from other countries in the literature as summarized in Table 7. The comparison shows that the levels of metals found in the present study are within the ranges of levels of metals reported in the literatures except Mg, Mn and Cu which are lower than the literature values. The level of toxic metal Cd in this study is above the literature cited. However, there are variations in the levels of individual metal in the barley grain from different countries. The variations are expected due to differences in the soil properties, geographical locations, climatic conditions and agricultural practices in different countries.

Table 7. Comparison of macro-essential, micro-essential and non-essential metals concentration (mg/kg, dry weight basis) in barley grain with reported values.

K	Mg	Ca	Mn	Fe	Cu	Zn	Pb	Cd	Country	Reference
3050	1650	1300	13	87	7.6	175	NR	NR	Egypt	[40]
8285	2686	891	23.9	937	8.12	33.7	1.45	0.29	Pakistan	[41]
NR	NR	NR	38.7	1546	10.4	53	1.21	0.5	Pakistan	[42]
3505	940	370	14.6	56.9	6.48	24.6	NR	NR	Poland	[43]
1822	1810	1160	12.3	82.9	9.2	69	NR	NR	Saudi Arabia	[44]
2700	650	200	NR	30	NR	21	NR	NR	UK	[45]
NR	NR	NR	1.67	31.85	0.15	3.85	0.03	ND	Ethiopia	[29]
NR	NR	NR	NR	NR	3.75	18.8	0.28	0.2	Iran	[6]
6066	574	462	8.54	227	1.22	47.9	1.24	3.73	Ethiopia	This study

NR = not reported, ND = not detected.

Analysis of variance (ANOVA)

One-way ANOVA was applied to assess the sources of variations observed in the level of metals as to whether it came from experimental procedure or due to heterogeneity among the samples. The results showed that there is a significant difference in the mean values of all the metals in the barley grain collected from four sampling area of Ethiopia. The differences are most likely due to heterogeneity among the samples because of the differences in the geographical locations and climatic conditions of the sampling sites.

Pearson correlation of metals in barley grain

Pearson correlation coefficients were employed to correlate the level one metal over the other metal. Pearson correlation coefficients revealed that there is negative, weak and/or moderate positive correlation between metals with each other. It was found that there was a strong positive correlation of Cu with Zn and Pb in the barley grain. The results are given in Table 8. These strong correlations may be due to their common natural sources as well as from similarity in their chemical properties.

Daily intake of minerals from barley

Metal concentrations in barely (this study), the amount that a person can get from 200 g barley per day, RDI and upper limit values of metals recommended by experts and agencies for a normal adult man is given in Table 9.

Table 8. Pearson correlation of metals within barely grain samples.

	K	Mg	Ca	Mn	Fe	Cu	Zn	Pb	Cd
K	1.0000								
Mg	0.6924	1.0000							
Ca	-0.9450	-0.4635	1.0000						
Mn	-0.7544	-0.5262	0.8378	1.0000					
Fe	0.2734	-0.1281	-0.2102	0.3575	1.0000				
Cu	0.6990	0.9472	-0.4301	-0.3240	0.1782	1.0000			
Zn	0.6325	0.9448	-0.3491	-0.2666	0.1431	0.9959	1.0000		
Pb	0.6051	0.9501	-0.3187	-0.2613	0.1010	0.9911	0.9988	1.0000	
Cd	0.9334	0.8984	-0.8054	-0.7598	0.0316	0.8510	0.8090	0.7971	1.0000

Table 9. Metal concentrations in barley (this study), the amount that a person can get from 200 g barley per day, RDI and upper limit values of metals recommended by experts and agencies for a normal adult person [4, 46-48].

Metal	Concentration in barley (mg/kg)	Amount of metal a person can get from 200 g barley (mg)	Daily recommended intake (RDI) (mg)	Maximum permissible limit (mg/day)
K	5482-6516	1096-1303	4700	ND
Mg	546-643	109-127	320-420	750
Ca	445-684	89-137	1000-1200	2500
Mn	7.31-9.8	1.5-2	1.8-2.3	11
Fe	127-439	25-88	10-15	45
Cu	0.88-1.86	0.18-0.4	0.9-2.3	10
Zn	42.8-56.8	8.6-11	10-15	40
Pb	0.39-2.73	0.08-0.55	0.02-3 µg/kg bw/day	25 µg/kg bw/day
Cd	3.01-4.66	0.6-0.9	0.8 µg/kg bw/day	7 µg/kg bw/week

bw = body weight, ND = not established.

The amount of mineral intake by a person who consumes 200 g barley per day is shown in Table 9. The data shows that amount of major metals (K, Mg and Ca) a person can get from barley is lower than the daily recommended values. Therefore the person needs K, Mg and Ca from other sources. The amount of trace metals (Mn and Cu) that the man can get from barley is also below the required amount. Hence other diet is required for to get these metals up to the recommended values. The amount of Fe supply from barley for all the sample sites is very sufficient. The amount of Zn a person can get from barley is within the range of daily recommended intake from the Bahir Dar but not from other three sites. The values for Pb and Cd are above the allowable limits. The man must not consume large quantity of foods from barley regularly.

CONCLUSION

The levels of metals in barley grain and four types of barley processed foods were determined. The order of metals contents in the raw grain and processed foods were different from each other. This is due to the differences in food processing practices. Barley grain from the selected sites accumulated relatively higher levels of K (5482-6516 mg/kg) and Fe (128-439 mg/kg) among major and trace metals, respectively, and lower level of Cu (0.88-1.86 mg/kg). The ANOVA result at 95% confidence level suggested significant differences in the level of metals among the barley grain samples collected from the four different regions of Ethiopia. These differences may be due to the difference in the soil chemical composition and environmental conditions which control the degree of mineral absorption by barley plants. The barley grain and its processed foods are good sources of essential metals. However, the Pb and Cd contents in the barley grains are above the allowable limits. Therefore people should not consume large quantity of foods from barley regularly.

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