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LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN LINSEED (*LINUM USITATISSIMUM*) CULTIVATED IN ETHIOPIA

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ABSTRACT. The levels of essential and non-essential metals were determined in linseed (*Linum usitatissimum*) samples collected in November 2011 from five different sites (Bale, East Gojam, Shoa, South Wello and Tigray) in Ethiopia where its cultivation is common. A 0.5 g dried powdered linseed was digested with 2 mL of nitric acid (HNO_3), 1 mL of perchloric acid ($HClO_4$) and 1 mL of hydrogen peroxide (H_2O_2) at 270 ^oC for 2:30 hours and the levels of metals determined by flame atomic absorption spectrometer. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution. Recoveries of the spiked samples varied from 91% to 109%. The levels (mg kg^{-1}) of metals determined were in the ranges Na (242–614), K (6,494–6,755), Mg (2,679–3,118), Ca (540–744), Cr (13–30), Mn (17–28), Fe (198–242), Co (23–42), Ni (12–16), Cu (25–45), Zn (29–40), and Pb (12–32). Cd was below method detection limit. K and Fe were with the highest concentration from major and trace metals, respectively. Analysis of variance (ANOVA) at 95% confidence level indicated that there is significant difference in the levels of all metals between the five samples means except K and Ni. The results indicated that Ethiopian linseed is a good source of essential metals and free from the toxic metal Cd but not from Pb.

KEY WORDS: Linseed, *Linum usitatissimum*, Essential metals, Non-essential metals, Ethiopia

INTRODUCTION

Linseed (*Linum usitatissimum*) is an erect annual, forming a short taproot with fibrous branches which may extend 90-120 cm in light soils. Linseed stands fourth after mustard, sesame and groundnut in edible oil production of the world [1]. Linseed has been a traditional crop in Ethiopia and it is the second most important oil crop in production after niger (*Guizotia abyssinica*) in the higher altitudes [2]. Ethiopia is the 5th major producer of linseed in the world after Canada, China, United States and India [3].

The principal linseed growing regions in Ethiopia are located at altitudes between 1800 and 2800 meter above sea level (masl), although it occasionally grows at altitudes as low as 1680 masl or as high as 3430 masl [4]. Arsi, Bale, Chercher Mountains, Eastern Welega, Eastern Gojam, Tigray, Southeast Wello, and Shoa are the major areas of production and South Gondar, Kefa, Gemogofa and Illubabor are small-scale production areas in Ethiopia. Linseed is a major oilseed and rotation crop for barley in higher elevations of Arsi, Bale, Gojam, Gonder, Wello, Shoa and Welega. High yields of wheat, barley and tef can be obtained following linseed [5]. In Ethiopia, in terms of area and production, linseed occupies the second position among the oilseed crops next to niger. The area under this crop is about 177,000 hectares with an average yield of 970 kg ha^{-1} [6]. The average area of cultivation of linseed in Ethiopia by small holders was 186,000 hectares with 1.7% of the total crop production during the period 2003/04–2008/09 [6].

Linseed is a multi-purpose crop. Its' seeds containing about 36-40% of oil, have long been used in human and animal diets and in industry as a source of oil and as a basic component or additive of various paints or polymers. Recently, there has been a growing interest in the probiotic properties of flax and in its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders [7-9]. The beneficial effects are mostly due to flax lipids. Flax oil is the richest plant source of linoleic (omega-6) and linolenic (omega-3)

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polyunsaturated fatty acids, which are essential for humans since they cannot be synthesized in the organism and must be ingested in food [10].

Scientific research over the past decade all over the world is indicating health benefits of omega-3 type oils, lignin and other soluble fiber present in the flax seed/linseed. Some of the medicinal uses of linseed oil are: flax seed oil mixed with an equal quantity of limewater, known as a rectal oil, is an excellent applicant for burns and scolds, rectal injection of 60 mL of oil, given at night and morning has been recommended for piles, freshly extracted oil is used as a laxative in doses of 30 mL, linseed oil is a vehicle for irritant drugs, linseed tea is prepared by boiling one part of linseed with 20 parts of water until grains become soft (the tea is used as a demulcent in cough especially those forms due to irritation of pharynx and upper part of respiratory passage. It is also used as a demulcent drink in intestinal or urinary catarrhs), for the preparation of cough syrup mucilage of linseed (1 in 8 parts of water) is used, crushed linseed is used in the form of poultice to apply warmth and moisture locally for the relief of superficial or deep rooted inflammation (it is prepared by boiling 28 g linseed with 72 mL of water. The poultice may be sprinkled with boric acid previous to application. The poultice mass is enclosed in muslin, the surface of poultice may be smeared with oil to keep it adhering to skin), linseed/flax seed and its oil have anti-inflammatory action the treatment of arthritis, feeding linseed and its oil may be indicated in hyperlipidemia to decrease platelet aggregation and also to reduce and control atherosclerosis, thrombosis and myocardial infraction by reducing cholesterol and low density lipids. It also lowers blood pressure [11].

In some European countries (Bulgaria, Germany, Hungary and Czech Republic) there were some investigations indicating the metal contents besides oil, protein, fatty acid and fiber contents [12]. The research in these countries showed that linseed has recently gained popularity as a health food product. It has high levels of fatty acids and minerals, giving it characteristics beneficial for functional foods. The levels of ten minerals (K, Na, Mg, Ca, Mn, Fe, P, Cu, Zn and B) were also determined and showed significant variability between lines [12]. In another research carried out in Pakistan [13] at differently processed linseed flours, roasting has not significantly affected the mineral contents of the flaxseed except Fe, Cu and Zn. Partial de-fattening resulted in a significant increase in the mineral content of the flax seed flours. Based on the recommended daily intakes and on the values obtained in that study, it is clear that flaxseed flour could be important in contributing to the overall daily dietary intake of essential elements especially the micronutrients whose deficiency is widespread in Pakistan [13].

In a study carried out in Italy [14], the contents of minerals in 35 different medicinal plants including linseed were analyzed. According to this study the mineral contents differs from one another depending on soil composition and the climate in which the plant grows. In another study conducted on some medicinal plants being used as food condiments including linseed in South Asian region, their composition by different methods and metals content by FAAS have been determined [15].

In recent years, there has been a growing interest in monitoring trace elements and heavy metal contents in some spices and herbs widely cultivated and consumed in Turkey including Mn, Fe, Cu, Zn and Cd, by ICP-AES. In that study the highest levels of metals determined in linseed were Zn (28 mg kg^{-1}) and Cd (0.13 mg kg^{-1}) and the concentration of the other metals was intermediate. The results of that study revealed that trace metal contents of some selected spices plants commonly cultivated in Turkey were within the low range [16].

Very recently, there has been a growing interest in monitoring trace elements and heavy metal contents in some spices and herbs widely cultivated and consumed in Ethiopia. These studies include levels of selected metals in the leaves of different species of thyme (*T. schimperi* and *T. vulgaris*) [17], metallic nutrients in enset (*Ensete ventricosum*) corm [18], levels of major and trace metals in onion (*Allium cepa* L.) [19], levels of metals in vegetables [20], levels of essential and non-essential metals in *Rhamnus prinoides* (Gesho) [21], mineral composition of Sorghum Landrace Accessions from Ethiopia [22], mineral contents of fruits of cactus pear

(*Opuntia ficus indica*) [23] and levels of major, minor and toxic metals in tubers and flour of *Dioscorea abyssinica* [24]. Mineral contents of seed and seed oils of Capparis species growing wild in Turkey have been reported during this year [25].

However, there is no research conducted in Ethiopia that is relevant to essential and non-essential metals or related to minerals content in linseed, even though Ethiopia is the 5th major producer of linseed. Therefore, the present study aimed to: (i) determine the concentrations of major (Na, K, Mg, Ca), trace (Cr, Mn, Fe, Co, Ni, Cu, Zn), and toxic (Cd, Pb) metals in linseed; (ii) compare the levels of metals in linseed samples from the different regions of Ethiopia; and (iii) compare the levels of metals in linseed from Ethiopia with literature data.

EXPERIMENTAL

Equipments

Electronic blending device (Moulinex, France) was used for grinding and homogenizing the sample. A 100 mL round bottom flasks fitted with reflux condensers were used in Kjeldahl (UK) apparatus hot plate to digest the dried and powdered linseed samples. Borosilicate volumetric flasks (50 and 100 mL) were used during dilution and preservation of samples and preparation of metals standard solutions. Volumetric pipettes (Pyrex, USA) with 1, 2, 5 and 10 mL capacity were used for measuring oxidizing reagents used for optimization of digestion procedure and preparation of samples as well as intermediate standards. Flame atomic absorption spectrophotometers (Buck Scientific Model 210 VGP AAS, East Norwalk, USA) equipped with deuterium arc back ground connectors and hollow cathode lamps with air-acetylene flame was used for the analysis of the analyte metals (K, Na, Mg, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) in the samples.

Reagents and chemicals

HNO_3 (69-72%), $HClO_4$ (60%) (BDH Laboratory Supplies AnalaR®, Poole England) and extra pure H_2O_2 (30%) (Scharlau Chemie S.A., European Union, Spain) were used for digestion of linseed samples. Lanthanum(III) nitrate hexahydrate ($La(NO_3)_3.6H_2O$) (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^{-1}, in 2% HNO_3, 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 in the spiking experiments. Deionized water was used for dilution of sample and intermediate metal standard solutions prior to analysis and rinsing glassware.

Sample site description, collection and transportation

Efforts were made to ensure representativeness in Ethiopian context by collecting samples from the most widely cultivated areas in Ethiopia in November 2011. The geographical locations of these sampling sites lie between the latitude 7°1′-14°15′N, longitude 37°43′-40°40′E, 1750-2840 masl and within the distance 809 km from Addis Ababa (capital city of Ethiopia) in all directions.

Recently cultivated linseed samples were collected from five major cultivation sites: Bale (Ginir, Goba and Robe, Oromia Region), East Gojam (Bichena, Debre Markos and Mota, Amhara Region), Shoa (Debre Birhan, Fiche and Hossaina, Amhara Region, Oromia Region and SNNPRS, respectively), South Wello (Dessie, Kombolcha and Wegeltena, Amhara Region) and Tigray (Adwa, Mekele and Wukro, Tigray Region). From a particular main site, three sub-sites were selected for the purpose of random sampling. About 500 g of the sample was taken

from each sub-sites and then mixed in to a single polyethylene plastic bags to get 1.5 kg of one bulk sample. The collected samples were packed into polyethylene plastic bags, labeled and transported to laboratory for further treatment.

Sample preparation

The linseed samples were washed with a running tap water to remove adsorbed soil particulates and then rinsed with deionized water. The samples were exposed to sun light for several days to reduce the moisture content and get constant weight so as to express the results in terms of dry mass basis. The dried linseed was ground using electronic blender and sieved (using 0.5 mm sieve) to prepare fine powder of linseed for digestion. Then the fine powder of linseed samples was kept in properly washed, dried and cleaned polyethylene plastic bags until appropriate amounts of the samples were taken for 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. Based on this fact the optimized condition for sample preparation in this study was (2 mL HNO_3 : 1 mL H_2O_2 : 1 mL $HClO_4$) volume ratio of reagents, 270 ^oC digestion temperature and 2:30 hour digestion time.

Digestion of samples

Applying the optimized conditions mentioned above, 0.5 g of powdered linseed samples were transferred into a 100 mL round bottom flask. Then 4 mL of a mixture of HNO_3 (69-72%), $HClO_4$ (60%) and H_2O_2 (30%) with a volume ratio of 2:1:1 (v/v) was added and the mixture digested on a Kjeldahl digestion apparatus fitted with a reflux condenser by setting the temperature first to 30 ºC for 10 min, second to 60 ºC for 10 min, third to 120 ºC for 10 min and then raising to 270 ºC for the remaining 2 hours. The digest was allowed to cool to room temperature for 30 min without dismantling the condenser and for 10 min after removing the condenser. To the cooled solution 15 mL of deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with Whatman, (110 mm and 125 mm, diameter) filter paper into 50 mL volumetric flask. The round bottom flask was rinsed subsequently with 5 mL deionized water until the total volume reached around 45 mL. To this final solution, about 0.67 g of $La(NO_3)_3.6H_2O$ was added and the solution was filled to the mark (50 mL) with deionized water. This addition of $La(NO_3)_3.6H_2O$ prevents the precipitation of Ca^{2+} and Mg^{2+} with the SO_4^{2-} and PO_4^{3-} (in which the anions may be formed from incomplete removal of the elements P and S from organic matter). As such the process of FAAS is element specific and the above addition makes Ca^{2+} and Mg^{2+} to be free for atomization and the SO_4^{-2} and PO_4^{-3} probably present in the solution is precipitated.

The digestion was carried out in triplicate for each bulk sample. Digestion of a reagent blank was also performed in parallel with the linseed 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 by FAAS.

Determination of metals in linseed samples

Calibration metal standard solutions were prepared for each of the metals from an intermediate standard solution containing 10 mg L^{-1} which was prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg L^{-1}. These secondary standards were diluted with deionized water to obtain four working standards for each metal of interest. Then K, Na, Mg, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb were analyzed with FAAS equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Three replicate determinations were carried out on each sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. All thirteen metals were determined by absorption/concentration mode and the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in the digested blank solutions.

Instrument calibration

The FAAS was calibrated using four series of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediate standard solutions (10 mg L^{-1}). The calibrated instrumental operating conditions including its determined method detection limit are shown in Table 1. The correlation coefficient in Table 1 for each metals shows that the change in absorbance with concentration is in good correlation.

Table 1. The wavelength, method detection limit, correlation coefficient, and calibration curve equation for determination of metals using FAAS.

Metal	Wavelength (nm)	Method detection limit (mg g^{-1} dry weight)	Correlation coefficient	Calibration curve equation
K	766.5	0.007	0.9999	$Y = 0.00618X - 5.32 \times 10^{-6}$
Na	589.0	0.004	0.9999	$Y = 0.0729X + 1.35 \times 10^{-3}$
Mg	284.2	0.001	0.9999	$Y = 0.186X + 1.68 \times 10^{-3}$
Ca	422.7	0.001	0.9999	$Y = 0.00266X - 7.04 \times 10^{-5}$
Cr	357.9	0.002	0.9998	$Y = 0.0101X + 4.18 \times 10^{-4}$
Mn	279.5	0.001	0.9992	$Y = 0.0238X - 2.97 \times 10^{-4}$
Fe	248.3	0.003	0.9999	$Y = 0.00412X + 7.15 \times 10^{-5}$
Co	240.7	0.006	0.9999	$Y = 0.00341X + 6.35 \times 10^{-5}$
Ni	232.0	0.003	0.9997	$Y = 0.00699X - 1.27 \times 10^{-5}$
Cu	324.7	0.003	0.9999	$Y = 0.0113X - 6.86 \times 10^{-5}$
Zn	213.9	0.001	0.9999	$Y = 0.116X - 1.70 \times 10^{-5}$
Cd	213.9	0.001	0.9999	$Y = 0.0671X + 6.27 \times 10^{-4}$
Pb	283.2	0.005	0.9999	$Y = 0.0671X + 6.27 \times 10^{-4}$

Method detection limits (MDL)

The MDL is typically determined to be in the region where the signal-to-noise ratio is greater than 3 but not necessarily quantified as an exact value. It can be calculated by multiplying the pooled standard deviation of the reagent blank (S_{blank}) by three (MDL = 3 x S_{blank}, n = 18). Method detection limits of the metals of interest are given in Table 1. The smaller values for

MDL indicate that the presence of trace amounts of metals of interest in the sample can be detected by the method.

Method validation

The validity of the optimized procedure was assessed by spiking experiments. For this purpose standard solution of 1000 mg L^{-1} (from BDH Chemicals Ltd Spectrosol®, Poole England) was used and intermediate standards of 100 mg L^{-1} and 10 mg L^{-1} were prepared. Thus, spiking was done in three triplicate groups. In the first group 330 µL of 1000 mg L^{-1} of K was spiked in a flask containing 0.5 g sample. In the second group 205 µL of 1000 mg L^{-1}, 24 µL of 1000 mg L^{-1}, 64 µL of 1000 mg L^{-1}, 45 µL of 1000 mg L^{-1} and 65 µL of 100 mg L^{-1} of Na, Mg, Ca, Fe and Co, respectively, were spiked in a flask containing 0.5 g sample. In the third group 40 µL of 100 mg L^{-1}, 50 µL of 100 mg L^{-1}, 35 µL of 100 mg L^{-1}, 80 µL of 100 mg L^{-1}, 75 µL of 100 mg L^{-1}, 50 µL of 10 mg L^{-1} and 70 µL of 100 mg L^{-1} of Cr, Mn, Ni, Cu, Zn, Cd and Pd, respectively, were in the flask containing the same amount of sample as the previous.

The spiked and non-spiked samples were digested and analyzed in similar condition using optimized procedure before for sample analysis. The results of recovery analysis are shown in Table 2 and the percentage recoveries lies within the range 91–114%. The percentage recovery for linseed samples are between 90 to 110% (100 ± 10), which are within the acceptable range for all metals except Pb, for which a recovery of 114% was obtained. The higher recovery for Pb (114%) may be attributed to the contamination of the sample with different Pb potential sources such as Pb acid battery while transportation and contamination while storing by local farmers and sample preparation before analysis.

Table 2. Recovery test for the optimized procedure of linseed sample.

Metal	Concentration of sample (mg kg^{-1})	Amount spiked (mg kg^{-1})	Concentration of spiked sample (mg kg^{-1})	Amount recovered (mg kg^{-1})	Percent recovery (% R)
K	10,500 ± 43	660	11,105 ± 39	605 ± 58	92 ± 9
Na	220 ± 0.5	48	272 ± 0.9	52 ± 1.0	108 ± 2
Mg	2,744 ± 17	411	3,133 ± 25	389 ± 30	95 ± 7
Ca	689 ± 6	127	805 ± 10	116 ± 11.7	91 ± 9
Cr	33 ± 0.4	8	41.7 ± 0.6	8.7 ± 0.72	109 ± 9
Mn	26.4 ± 0.5	10	35.9 ± 0.6	9.5 ± 0.78	95 ± 8
Fe	138 ± 1.1	89	223 ± 1.5	85 ± 1.86	96 ± 2
Co	22.0 ± 0.9	13	34.7 ± 1	12.7 ± 1.3	98 ± 10
Ni	14 ± 0.5	7	21.6 ± 0.5	7.6 ± 0.71	109 ± 10
Cu	25 ± 0.8	16	42 ± 0.7	17 ± 1.06	106 ± 7
Zn	35.0 ± 0.7	15	48.9 ± 0.1	13.9 ± 0.71	93 ± 5
Cd	ND	1	1.92 ± 0.05	0.92 ± 0.05	92 ± 5
Pb	30 ± 0.9	14	46 ± 1	16 ± 1.3	114 ± 9

ND = not detected.

Data analysis

Origin 6.0 software was used for the preparation of calibration curves and the data analysis. One way ANOVA was used for the experimental design and to compare the mean values the metals between different sampling sites. Pearson correlation coefficient was used to determine the degree of positive or negative correlation between the metals.

RESULTS AND DISCUSSIONS

Levels of metals in linseed samples

The determination of metals level was carried out by FAAS and the accuracy and precision of the results was determined by different statistical methods as described in experimental section. Percent relative standard deviation (% RSD) all the results were in good range, i.e. all the values determined were in the range of mean value ($\overline{X}$) ± 10%. Mean values were determined from triplicate analysis of each sample and triplicate samples were used for each sample site. As such the mean values determined were triplicate of triplicate analysis for each metal and the results were in terms of mean values ($\overline{X}$) ± SD (where n = 9, for all the metals in this study). Among the analyzed metals the value for Cd was not detected because it was below method detection limit. Results are listed in terms of mean value and standard deviation (SD) of mg kg^{-1} dry weight bases and percent relative standard deviation in Table 3.

Table 3. Mean concentration ($\overline{x}$ ± SD, n = 9, mg kg^{-1} dry weight) of major, trace and toxic metals in each sample sites analyzed by FAAS.

Metal	Sampling site				
	Bale	East Gojam	Shoa	South Wello	Tigray
	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD
K	6522±71	6494±68	6755 ±78	6644±47	6539±61
Na	242±21	503±25	314±21	614±23	525±28
Mg	2740±28	3029±28	3013±23	3118±25	2679±23
Ca	635±18	622±6	624±20	744±8	540±9
Cr	16±0.4	13±0.9	30±1	27±0.6	33±1.8
Mn	23±0.6	28±1	22±1	22±1	17±0.5
Fe	198±2	232±0.8	242±9	201±0.7	216±7
Co	28±0.5	42±0.4	30±1	37±3.6	23±2
Ni	14±1.3	15±0.7	15±1.2	16±1.4	12±1.0
Cu	35±0.9	25±0.8	37±0.9	45±3.8	41±2
Zn	33±0.1	35±0.6	29±0.8	34±0.3	40±1
Cd	ND	ND	ND	ND	ND
Pb	32±2.5	12±1	17±1.5	28±1	22±2

ND = Concentration of the metal was below the method detection limit (< 0.001 mg g^{-1}).

Distribution patterns of metals in the samples

Metals uptake by plants may occur through different and complex biochemical processes. This uptake varies based on the ability of the plants to absorb metals from the soil, the availability of the mineral elements in soluble and absorbable forms, the abundance of specific metals at the specified site, and the contamination level of the soil with heavy metals. The variation of metals levels in soil arises because of increasing industrialization and associated pollution of the biosphere, use of different types of fertilizers, pesticide treatment, and others are the main contributors. The use of sewage sludge, pesticides, irrigation of waters and fertilizers on agricultural land has made some of that land of questionable quality for production of food for humans and animals. The distribution and accumulation of metals in linseed are the reflection of the mineral composition of the soil and environment in which linseed plant grows. Therefore, the actual metal content of linseed vary considerably according to geographic origin, the use of fertilizers with different chemical compositions and other characterizing features such as water for irrigation.

Concentration of macro-essential (major) metals in linseed

Plants accumulate metals from the soil and environment in different parts. The amount of metals accumulated in the plant varies in different edible parts. But this study focuses the level of metals in seed of linseed because the common edible part of it is its seed for human beings. There is a variation in the metal concentration of macro-essential metals among the sample sites. As the results in Table 3 show that highest concentration of K was within the range (6,494–6,755 mg kg^{-1} dry weight) in all sites followed by Mg within the range (2,679–3,118 mg kg^{-1} dry weight) which is the second with highest concentration next to K. Ca was the third in its concentration level followed by Na which is the forth one among the macro-essential minerals in most sample sites. In short the concentration profile of macro-essential metals determined in linseed was K > Mg > Ca > Na.

Among the sample sites highest concentration of K was determined in sample site Shoa (6,755 ± 78 mg kg^{-1} dry weight) followed by South Wello (6,644 ± 47 mg kg^{-1} dry weight) and least value at East Gojam (6,494 ± 68 mg kg^{-1} dry weight). In this study the concentration of K by sample sites decreases in the order Shoa > South Wello > Tigray > Bale > East Gojam (6,755 ± 78; 6,644 ± 47; 6,539 ± 61; 6522 ± 72 and 6,494 ± 68 mg kg^{-1} dry weight, respectively). The one with highest concentration of Na was in the sample site South Wello (614 ± 23 mg kg^{-1} dry weight) followed by Tigray (525 ± 28 mg kg^{-1} dry weight) and least amount of Na was in sample site Bale (242 ± 21 mg kg^{-1} dry weight). Concentration decrement by sample sites can be arranged according to the order South Wello > Tigray > East Gojam > Shoa > Bale (614 ± 23, 525 ± 28, 503 ± 25, 314 ± and 242 ± 21 mg kg^{-1} dry weight, respectively). The variation for Na level in linseed by sample site is somewhat wide and this may be attributed to the sample site distance from sea or lakes. As such the nearer the cultivation land to the lake, sea or ocean; the more amount of Na in that soil and the more amount can be absorbed by the plants [26, 27]. Similarly highest amount of Mg and Ca (3,118 ± 25 and 744 ± 8 mg kg^{-1} dry weight, respectively) and lowest amount (2,679 ± 23 and 540 ± 9 mg kg^{-1} dry weight, respectively) were determined in sample sites South Wello and Tigray, respectively. The decreasing order for Mg and Ca by sample sites are South Wello > East Gojam > Shoa > Bale > Tigray and South Wello > Bale > Shoa > East Gojam > Tigray, respectively. The levels of K, Mg and Ca in linseed did not show much variation by sample sites.

The higher levels of K and Mg in linseed is probably due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue. The other probable reason for higher concentration of K and Mg is if the soil which have been used for cultivating the plant, are highly fertilized with manure and organic residues, they were high in available K and Mg. Hence, the plant has high amount of these metals.

Concentration of micro-essential (trace) metals in linseed

As shown in Table 3 Fe (198–242 mg kg^{-1} dry weight) was the most accumulated trace metal followed by Cu (25–45 mg kg^{-1} dry weight), Co (23–42 mg kg^{-1} dry weight) and Zn (29–40 mg kg^{-1} dry weight) in linseed sample. However, the concentration values of these trace metals indicates that the concentration ranges of trace metals overlap each other among the sample sites except Fe in which its value is higher in all sample sites and Ni (12–16 mg kg^{-1} dry weight) in which lowest concentration values was determined. As such highest concentration of Fe was determined in sample site Shoa (242 ± 9 mg kg^{-1} dry weight) followed by East Gojam (232 ± 0.8 mg kg^{-1} dry weight) and lowest in the sample site Bale (198 ± 2 mg kg^{-1} dry weight). The result for Fe in linseed indicates that the variation by sample site is very narrow. In general the concentration profile of Fe decreased by sample site was arranged in the order Shoa (242 ± 9 mg

kg^{-1} dry weight) > East Gojam (232 ± 0.8 mg kg^{-1} dry weight) > Tigray (216 ± 7 mg kg^{-1} dry weight) > South Wello (201 ± 0.7 mg kg^{-1}) > Bale (198 ± 2 mg kg^{-1}).

The pattern of concentration of trace metals in linseed collected from sample site Bale was decreased as Fe >> Cu > Zn > Co > Mn > Cr > Ni. Similarly the concentration profile according to this work was decreased in the order for sample site East Gojam (Fe >> Co > Zn > Mn > Cu > Ni > Cr), Shoa (Fe >> Cu > Cr ≈ Co ≈ Zn > Mn > Ni), South Wello (Fe >> Cu > Co > Zn > Cr > Mn > Ni) and Tigray (Fe >> Cu ≈ Zn > Cr > Co > Mn >Ni).

Consideration of individual metal by sample site indicates that the trend for Cr decreased by the order Tigray ≈ Shoa > South Wello > Bale > East Gojam. For Mn the trend was almost opposite to that of Cr as East Gojam > Bale > Shoa ≈ South Wello > Tigray. The trend for other trace metals was briefly arranged as for Co (East Gojam > South Wello > Shoa > Bale > Tigray); for Ni (South Wello ≈ Shoa ≈ East Gojam ≈ Bale > Tigray); for Cu (South Wello > Tigray > Shoa > Bale > East Gojam) and for Zn (Tigray > East Gojam ≈ South Wello ≈ Bale > Shoa) based on this study of metals analysis by FAAS. The result for levels of trace metals in linseed indicates that the variation by sample site is very narrow.

The concentration values shown in Table 3 also show that the variations in concentrations of trace metals is not as much significant and comparable to each other. For example the concentration of Ni varies with in small range (12–16 mg kg^{-1} dry weight) and implies that the composition of Ni was invariant in comparison to other metals.

Results in this work for metal levels also indicate that the higher the concentration of the metal, the higher the variation in metal content by sample sites. Thus, the variation for Fe by sample sites is higher because Fe content in the sample was highest among the micro-essential metals and the variation for Ni by sample sites was smaller due to its lower content.

Concentration of non-essential (toxic) metals in linseed

Dietary exposure to Cd is estimated to be about 1.2 x 10^{-4} to 4.9 x 10^{-4} mg kg^{-1} of body weight daily. Intake of dietary Cd should not exceed 0.007 mg kg^{-1} of body weight, per week, according to the World Health Organization. For a healthy male of about 80 kg of body weight, that is only 0.56 mg per week, a very small amount indeed. Cd is retained in the kidneys and liver (50 to 70 percent of accumulated Cd is deposited in these organs); excessive exposure can lead to kidney disease and serious liver damage. Possible effects of intense Cd exposure include emphysema, bone disorders such as osteoporosis and osteomalacia, cancer, and a shortened life span. However, the levels of Cd in this work was below method detection limits (< 0.001 mg g^{-1}) and using linseed for human nutrition may not affect human health due to the accumulation of Cd from linseed.

Pb is a major chemical pollutant of the environment, and is highly toxic to man. Pb can cause brain and kidney damage, decrease in hemoglobin production and male fertility. It enters human body by inhalation and ingestion, absorbed and carried by the blood; it is accumulated in liver, kidney, and bone up to about the fifth decade of life. Pb causes brain damage particularly to the young. There is evidence that Pb pollution can induce aggressive behavior in animals which can also occur in humans.

The values determined for Pb level in this work are presented in Table 3. As we can see from the table highest amount of Pb was determined from sample site Bale (32 ± 2.5 mg kg^{-1} dry weight) followed by South Wello (28 ± 1 mg kg^{-1} dry weight) and least concentration in sample site East Gojam (12 ± 1 mg kg^{-1} dry weight). Comparison of the Pb concentration by sample site decreased in the order Bale > South Wello > Tigray > Shoa > East Gojam. The variation for Pb content in linseed by sample site may be attributed to exposure to contamination during storage and transportation by cultivators.

Comparison of metal levels of the present study with literature values

Comparison of analytical data with reference material is a common practice in analytical chemistry to validate the results. However, there is no standard reference material to do so and the determined results should be compared with the investigations made in other countries by other investigators. Earlier studies on linseed were made in different countries, in Ethiopia in particular, focused on the essential oil composition and biological aspects. Studies on the level of major, trace and toxic metal composition of the plant in Ethiopia was not carried out.

The comparative values of the results for macro-essential metals in this study with that was reported in the literatures were shown in Table 4. As the results in the table revealed, the levels of macro-essential metals determined in this work are in good agreement with other researchers' work that have been carried out in different countries and regions. For example the results in this study for these metals levels are almost similar with that reported by Kiralan *et al*. [12] from European Union, Hussain *et al*. [13] from Pakistan, Donatella *et al*. [14] from Italy and Iftikhar *et al*. [15] from south Asian regions. But the results in this study are higher than that reported by Khan *et al*. [28] from Pakistan for all macro-essential metals.

As shown in Table 4, the concentration of K (6,494–6,755 mg kg^{-1} dry weight) determined in this study is in good agreement with other researchers like Kiralan *et al*. [12] (8,770 mg kg^{-1} dry weight) and Donatella *et al*. [14] (8,820 mg kg^{-1} dry weight). However, the result in this study for K level is to some extent higher than that reported by Iftikhar *et al*. [15] (2,736 mg kg^{-1} dry weight) in South Asian regions and much lower than that determined by Hussain *et al*. [13] (13,690 mg kg^{-1} dry weight). The results in this work also shows that K is the one with highest concentration among macro-essential metals as well as trace and heavy metals which is in agreement with that reported by all researchers in their work except that reported by Iftikhar *et al*. [15] (2,736 mg kg^{-1} dry weight) in which it is the second one next to Na. In general, the results in this work for K levels are within the range of values determined in the literatures.

Similarly the levels of Na determined in this work (242–614 mg kg^{-1} dry weight) is somewhat in good agreement with that reported by Kiralan *et al*. [12] (689 mg kg^{-1} dry weight) and Hussain *et al*. [13] (582 mg kg^{-1} dry weight). But the result in this study is much lower than that reported by Iftikhar *et al*. [15] (5,005 mg kg^{-1} dry weight). The Mg content of linseed determined in this study (2,679–3,118 mg kg^{-1} dry weight) is higher than that reported by Donatella *et al*. [14] (1,067 mg kg^{-1} dry weight) and Iftikhar *et al*. [15] (126 mg kg^{-1} dry weight), almost comparable to that reported by Kiralan *et al*. [12] (3,480 mg kg^{-1} dry weight) and much lower than that reported by Hussain *et al*. [13] (7,130 mg kg^{-1} dry weight). The level of Ca of this study (540–744 mg kg^{-1} dry weight) is comparable to that reported by Iftikhar *et al*. [14] (658 mg kg^{-1} dry weight), but much lower than those determined by Kiralan *et al*. [12] (1,858 mg kg^{-1} dry weight), Hussain *et al*. [13] (3,980 mg kg^{-1} dry weight) and Donatella *et al*. [14] (1,930 mg kg^{-1} dry weight).

Table 4. Comparison of macro-essential metals concentration, (mg kg^{-1}, dry weight basis) in linseed samples with reported values.

Concentration of metal (mg kg^{-1})				Country	Reference
K	Na	Mg	Ca		
8,770	689	3,480	1,858	Europe	[12]
13,690	582	7,130	3,980	Pakistan	[13]
8,820	NR	1,067	1,930	Italy	[14]
2,736	5,005	126	658	South Asia	[15]
6,494–6,755	242–614	2,679–3,118	540–744	Ethiopia	This study

NR = not reported.

The levels of micro-essential metals in this study is almost similar with those reported in literatures except Cr and Fe in which the values determined here are to some extent higher than

those values reported in the literature. The comparative results determined in this study with literature values are shown in Table 5.

The level of Cr according to this work is within the range (13–30 mg kg^{-1} dry weight) which is higher than that reported by Iftikhar *et al*. [15] (<2 mg kg^{-1} dry weight). The higher value for Cr level may not be concluded unless enough data from literatures are accessed. For Mn the concentrations determined (17–28 mg kg^{-1} dry weight) are almost similar (within the same range) with those reported from other countries. Fe in this study (198–242 mg kg^{-1} dry weight) as well as those reported by others in the literatures is the one with higher concentration among trace metals however the values determined here are higher than others' determinations in the literature. It is also the one with highest concentration among all metals determined and reported by Khan *et al*. [28] (51 mg kg^{-1} dry weight) but not the case in this work. As researches in the recent years in Ethiopia indicated that the higher level of Fe in this study in comparison with other literature values may be attributed to the fact that the soil in Ethiopia is rich with Fe content. The concentration of Co and Ni determined in this work (23–42 mg kg^{-1} and 12–16 mg kg^{-1} dry weight, respectively) is within the ranges reported by Donatella *et al*. [14] (<1 mg kg^{-1} and 0.8 mg kg^{-1} dry weight, respectively) to Iftikhar *et al*. [15] (76 mg kg^{-1} and 19 mg kg^{-1} dry weight, respectively). The mean concentrations of Cu in present study (25–45 mg kg^{-1} dry weight) is in good agreement with those reported by Hussain *et al*. [13] (35 mg kg^{-1} dry weight) and Iftikhar *et al*. [15] (25 mg kg^{-1} dry weight), but a little bit higher than the values determined by Kiralan *et al*. [12] (12 mg kg^{-1} dry weight), Khan *et al*. [28] (4.7 mg kg^{-1} dry weight) and Donatella *et al*. [14] (15 mg kg^{-1} dry weight). Zn concentration in this work (29–40 mg kg^{-1} dry weight) is also similar with other researchers like Kiralan *et al*. [12] (34 mg kg^{-1} dry weight), Donatella *et al*. [14] (42 mg kg^{-1} dry weight) and Özkutlu *et al*. [16] (28 mg kg^{-1} dry weight); somewhat lower from the value reported by Hussain *et al*. [13] (79 mg kg^{-1} dry weight) and higher from the amount determined by Khan *et al*. [28] (14 mg kg^{-1} dry weight).

The determination for levels of toxic metals was also carried out in this study and Cd was the one below method detection limit (<0.001 mg g^{-1}). However, value as high as 0.13 mg g^{-1} dry weight is reported in literature from Turkey by Özkutlu *et al*. [16]. This implies the amount of Cd present in linseed is very small and using linseed for different feeding purposes may not affect human health, because of Cd over dosage is less. The level of Pb in this work (12–32 mg kg^{-1} dry weight) revealed that, it is higher than those reported by other researchers (Donatella *et al*. [14] and Iftikhar *et al*. [15] as presented in Table 5. Using linseed for feeding continuously may lead to toxic effects like brain and kidney damage, decrease in hemoglobin production and male fertility based on the values determined in this work. However, further investigation concerning Pb in linseed with other alternative methods and large sample size should be recommended to come up with a firm conclusion.

Table 5. Comparison of micro-essential and heavy metals concentration, (mg kg^{-1}, dry weight basis) in linseed samples with reported values.

Concentration of metal (mg kg^{-1})									Country	Reference
Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb		
NR	30	76	NR	NR	12	34	NR	NR	Europe	[12]
NR	47	NR	NR	NR	35	79	NR	NR	Pakistan	[13]
NR	8.3	51	NR	NR	4.7	14	NR	NR	Pakistan	[28]
NR	16	72	<1	0.8	15	42	NR	<0.1	Italy	[14]
<2	7.7	NR	76	19	25	NR	NR	1.7	South Asia	[15]
NR	NR	NR	NR	NR	NR	28	0.13	NR	Turkey	[16]
13–30	17–28	198–242	23–42	12–16	25–45	29–40	ND	12–32	Ethiopia	This study

NR = not reported. ND = not detected.

In general, the concentrations of metals observed are more or less comparable with the reported literature values. However, relatively higher concentrations of Cr and Fe from essential

metals and Pb from non-essential metals are observed in this study in comparison to the reported values.

Table 6. Analysis of variance (ANOVA) between and within linseed samples at 95% confidence level.

Metal	Comparison	SD (mg kg^{-1})	Df	F_{cal}	F_{crit}	Remark
K	Between samples	108	4	2.60	2.61	No significant difference between sample means
	Within samples	67.0	40			
Na	Between samples	155	4	43.1	2.61	Significant difference between sample means
	Within samples	23.6	40			
Mg	Between samples	194	4	58.3	2.61	Significant difference between sample means
	Within samples	25.4	40			
Ca	Between samples	72.8	4	35.6	2.61	Significant difference between sample means
	Within samples	12.2	40			
Cr	Between samples	9.10	4	93.7	2.61	Significant difference between sample means
	Within samples	0.94	40			
Mn	Between samples	3.91	4	22.7	2.61	Significant difference between sample means
	Within samples	0.82	40			
Fe	Between samples	19.1	4	24.0	2.61	Significant difference between sample means
	Within samples	3.90	40			
Co	Between samples	7.52	4	25.1	2.61	Significant difference between sample means
	Within samples	1.50	40			
Ni	Between samples	1.52	4	1.84	2.61	No significant difference between sample means
	Within samples	1.12	40			
Cu	Between samples	7.54	4	20.1	2.61	Significant difference between sample means
	Within samples	1.68	40			
Zn	Between samples	3.96	4	50.0	2.61	Significant difference between sample means
	Within samples	0.56	40			
Pb	Between samples	8.10	4	25.6	2.61	Significant difference between sample means
	Within samples	1.60	40			

SD = standard deviation, Df = degree of freedom, F_{cal} = F calculated, F_{crit} = F critical.

Pearson correlation

In this study, to correlate the effect of one metal concentration on the concentration of the other metal, the Pearson correlation matrices using correlation coefficient (r) for the samples were used and presented in Table 7. The values of Pearson correlation coefficient revealed that there is weak and/or moderate positive or negative correlation of metals with each other except for some metals. The weak negative or positive correlation indicating that the presence or absence of one metal affect in lesser extent to the other. Some exception observable from the table is that, there is high positive correlation for K with Cr, Mg with Co and Ni, Ca with Ni, Cr with Cu, Mn with Co and Co with Ni; which may arise from common anthropogenic or natural sources as well as from similarity in chemical properties. The high negative correlation between Fe and Pb indicate that large absorption of Fe may affect the absorption of Pb in linseed plant.

Table 7. Pearson correlation matrices for metals in linseed sample (n = 5).

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Pb
K	1											
Na	-0.12	1										
Mg	0.48	0.37	1									
Ca	0.32	0.25	0.75	1								
Cr	0.70	0.23	-0.01	-0.07	1							
Mn	-0.23	-0.10	0.56	0.35	-0.81	1						
Fe	0.38	-0.11	0.30	-0.38	0.14	0.25	1					
Co	-0.09	0.38	0.82	0.59	-0.53	0.86	0.18	1				
Ni	0.42	0.12	0.93	0.88	-0.19	0.64	0.07	0.79	1			
Cu	0.47	0.29	-0.06	0.29	0.80	-0.79	-0.47	-0.48	-0.07	1		
Zn	-0.68	0.57	-0.53	-0.46	0.03	-0.41	-0.32	-0.25	-0.68	0.12	1	
Pb	-0.06	-0.19	-0.35	0.36	0.07	-0.40	-0.91	-0.41	-0.07	0.61	0.04	1

CONCLUSSION

In this study metal levels in linseed from five different regions in Ethiopia were analyzed for their contents of K, Na, Mg, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb using flame atomic absorption spectrometer. The optimized wet digestion method for linseed analysis was evaluated through the recovery experiment and a good percentage recovery was obtained (100 ± 10) for all the metals identified. The levels of essential metals in linseed determined in this study varied in the order K (6494–6755 mg kg^{-1}) > Mg (2679–3118 mg kg^{-1}) > Ca (540–744 mg kg^{-1}) > Na (242–614 mg kg^{-1}) > Fe (198–242 mg kg^{-1}) > Cu (25–45 mg kg^{-1}) > Ni (12–16 mg kg^{-1}) and approximately overlapping ranges for the other metals. The non-essential heavy metal, Cd, was found to be below the method detection limit. The results of this work indicated that linseed accumulates relatively higher amounts of K and Pb among the determined essential and non-essential metals, respectively. The contents of minerals in linseed in this study were within the daily recommended level and thus advisable as healthy food for treatment of different health complications.

Statistical analysis by using one way ANOVA indicated that there is significant difference in mean concentration of metals between sampling sites except K and Ni. This may be attributed to differences in soil composition, use of different fertilizers, pesticides, and quality of irrigation water. For K and Ni, the difference may only be attributed to random errors in the experimental procedures. The concentration of Pb determined in this work is somewhat higher from other experimental results using the same method. In general the metal contents in linseed in Ethiopia are comparable to that reported in the rest of the world.

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