2. Determination of Selected Essential and Non-Essential Metals in Black Mustard (Brasicanigra) Seed cultivated in Amhara Region, Ethiopia

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ABSTRACT

Brasicanigra, with the local name of "senafich", is one of the crops grown in Ethiopia. In this study, we examined the level of some metals in this seed, which is cultivated in different parts of east Gojjam Amhara region, Ethiopia. The levels of the metals were determined using Flam Atomic Absorption Spectroscopy (FAAS) after digesting the powdered Brasicanigra samples with HNO_3, $HClO_4$ and H_2O_2 mixture for 2:30 h*. The study found out that the level of metals in the seed was: Ca (22.1 to 27.16 mg/100 g), Mg (13.74 to 18.71 mg/100g), Fe (8.80 to 9.267 mg/100 g), Zn (3.243 to3.333 mg/100 g), Cu (1.925 to 2.066 mg/100g) and Pb (0.087 to 0.181 mg/100 g). A statistical analysis of variance (ANOVA) was also run to see the geographical variation of metal concentration in the seeds. Accordingly, the effects of geographical location on Ca and Cu were significantly different among different areas while significant difference was not observed among other metals, i.e. Mg, Zn, Fe and Pb. In general, the content of metals in Brasicanigra was foundas: Ca> Mg > Fe > Zn > Cu >Pb. The result of this study showed that Brasicanigra has good source of Ca, Mg, Fe, Cu and Zn for human beings. However, the concentration of Pb was found to be higher in all the studied samples as compared to the WHO standards. Thus, further studies should be required so as to re-confirm the data. In general, Brasicanigra is rich in essential metals and safe to consume and could be an alternative source of essential metals to the individual daily intake.*

***Keywords*:** *Brasicanigra, Essential metals, Non-essential metals, Flam Atomic Absorption Spectroscopy*

1 Introduction

Brassica nigra belongs to the botanical family Brassicaceae (Cruciferae). It is commonly called as "senaffich" in Amharic. It is an annual herbaceous plant. It grows up to 2 m (a little over 6 ft),

with many branches. Its stems are cylindrical with a very smooth surface. Its leaves are densely covered with hispid hairs, radical not greatly developed, pinnatifid or with very large terminal lobed. A single plant may produce thousands of seeds, which must be harvested by hand or mechanically before they fully ripen because the siliques spontaneously split and disperse the seeds when they are mature [1].

*Brassica nigra*seeds are roughly globular with a diameter of 1 to 1.5 mm and a dark brown colour; the seed coat is pitted and when soaked in water the seeds produce a strong pungent odour[2]. There are approximately 40 different varieties of mustard plants, but the three principal ones, which also vary in colour, are *Brassica alba* (yellow-white), *Brassica nigra* (black), and *Brassica juncea*(brown).

Black mustard brown mustard oriental mustard yellow mustard

Figure 1: Types of mustard seeds [2]

Brassica nigra has different functions since ancient time. Among ancient scholars, Pythagoras, for example, mentioned the use of mustard seeds for scorpion stings while Hippocrates used it for the preparation of medicines. The medicinal properties of mustard were also known to the Greeks and Romans, and ancient documents written by Cato, Columella and Pliny suggest that mustard seeds were cultivated and used as a condiment, mixing the ground seeds with wine ~~must~~ to make a paste, hence the name "mustard" [3]. Mustard seeds are also used to prepare food condiments and have wide range of applications in the food industry.

It is also believed that mustard is also used as a protein source, flavor enhancer and as a binder in the manufacturing of processed meats. The different mucilage contents in the different varieties of mustard allow the manufacturing of products with different viscosities. Its seed hulls are also used as a thickening agent and stabilizer in food preparation. The presence of sinigrin in the brown and

oriental varieties makes them suitable for the manufacture of hot mustard for remote markets and the production of mayonnaise. High oil content oriental mustard is used to meet the oilseed demand in the Indian subcontinent where one of its main uses is cooking oil production [4].

There are some studies conducted on this seed which was cultivated outside Ethiopia. For example, Krishnaveni and Saranya conducted a study on phytochemical characterization of *Brassica nigra* seeds. They found out that *Brassica nigra* seeds showed a positive result for all the phytochemicals tested. According to FT-IR, the functional groups present are aldehyde, ketone, amino acids and carboxylic acids. The GC-MS results showed highest peak for Octadecadienoic acid and Erucic acid [5]. Another study was conducted by Akkoyun, Dostbil and kiran. This study was made to determine the antimicrobial activity of mustard (*Brassica nigra L.*) The investigation showed the different levels of inhibitory activity to test strains while the mustard has inhibitory activity on the all test strains [6]. Tahrakan and Madhavan also tried to determine phytochemical screening, total phenolic quantification, antioxidant and anthelmintic activity of hot water extract of *Brassica nigra L*. They found out that hot water extract of *Brassica nigra* consists of phytochemicals like carbohydrate, amino acid, protein, alkaloid, steroids, saponins, phenols and tannins. The extract also confirmed DPPH radical scavenging activity [7].

There are also some studies which were conducted on this seed cultivated in Ethiopia. For example, Abraham, Eshetie and Getnet conducted a study to evaluate the antimalarial activity of 80 % methanolic extract of *Brassica nigra* against Plasmodium berghei infection in mice. They concluded that seed extract of *Brassica nigra* showed good chemosuppressive and moderate chemoprophylactic activities and the plant may contain biologically active principles which are relevant in the treatment and prophylaxis of malaria [8]. Amanuel, Atsede, Haftu and Nigisti conducted a research on antibacterial activity of oil extracts of *Brassica nigra* seeds against bacteria isolated from fresh juice in selected areas of Axum town. They found that *Brassica nigra* seed is important for antibacterial activity [9].

While the studies outside Ethiopia focused on extracting some organic components, many of the local studies focused on the antibiotic activity of the seed. Local studies, of course, suggested that further studies of the plant for its active components such as Fe, Zn and others should be conducted [8].

However, there are literatures about *brassica niga* and its few elements (Zn, Cu, Mn, Cr, Fe and Se). There is no report on the levels of essential and non-essentia metals in *brassica niga* cultivated in Ethiopia; particularly in the study area East Gojam, Amhara Regional State. Therefore, the objectives of this study were to determine the concentration of essential metals (Ca, Mg, Fe, Zn and Cu) and toxic metal (Pb) in *brassica niga* seed using Flame Atomic Absorption Spectroscopy (FAAS) and to compare the levels of metals in three Woredas (Gozamin, Awabel and Dejen) in East Gojam Zone, Amhara Region, Ethiopia.

2 Materials and Methods

2.1 Description of the study area

The study was carried out in selected sites of three different agricultural locations of East Gojjam Zone of the Amhara Regional State (Ethiopia) namely, Dejen, Awabel and Gozamen areas. These areas were selected due to *Brassicanigra* is dominantly cultivated and consumed.

Dejen is a town in west-central Ethiopia which is located in the East Gojjam Zone of the Amhara Region on the edge of the canyon of the Abay. It has a latitude and longitude of 10°10′N 38°8′E and an elevation between 2421 and 2490 meters above sea level.

The second Sample area, Awabel (Lumame), is situated in East Gojjam, Amhara region. Its geographical coordinates are 10° 15' 0" North, 37° 56' 0" East. The third sample area, Gozamin, is found in the *North* western highlands of Ethiopia at a geographical location of 10°1′ 46″ and 10° 35′ 12″ N latitudes and 37° 23′ 45″ and 37° 55′ 52″ E longitudes and at a distance of 300 and 265 km from Addis Ababa and Bahir Dar, respectively.

2.2 Chemicals and Reagents

All chemicals and reagents used in this research were analytical grade. Hydrogen peroxide (30%) (BDH Chemicals, England),(69-72%)HNO_3 (Spectrosol, BDH, England) and 70% $HClO_4$ (Aldrich, A.C.S. Reagent, Germany) were used for digestion of *Brasicanigra* samples. Lanthanum nitrate hydrate (98%, Aldrich, Muwaukee, USA) was used to avoid refractory interference or for releasing calcium and magnesium from their phosphates. Stock standard solutions containing 1000mg/L in 2% HNO_3 of the metals (Ca, Mg, Fe, Zn, Cu, and Pb) (Buck Scientific Puro-Graphictm) were used for preparation of calibration standards and in the spiking experiments.

Working standard solutions were obtained by suitable dilution from stock solution. De-ionized water was used for dilution of samples, intermediate and working metal standard solutions to analyze and to rinse glass wares.

2.3 Instruments and Equipment

The necessary apparatus and instruments used for this study were electronic beam balance (model ESJ200-4) with + 0.0001g precision for mass measurement. A drying oven (model DHG-9055A) was used to dry the washed seed samples. A refrigerator (West-point model WRES-358.x) was used for sample preservation after digestion and before AAS analysis. Micro pipettes (10-100 µL and 100-1000 µL) were used for measuring reagents used for the preparation of standard solutions. BUCK SCIENTIFIC MODEL 210 VGP Atomic absorption spectrophotometer equipped with deuterium arc background correctors were used for analysis of the analyte metals using air-acetylene flame. Different size (50 mL, 100mL and 1000 mL) volumetric flasks were used during dilution. Whiteman 110mm filtrate papers were used for filtration of sample solution after digestion.

2.4 Sample Collection and pre-treatment of *Brasicanigra* seeds

Commercially available seed of black mustard (*Brassica nigra*) was purchased from local farmer of Dejen, Awable and Gozamen Woredas. The sampling techniques were random sampling. The samples were packed into Polyethylene plastic bags, labelled and transported to the laboratory for further analysis.

The Samples were sorted out to remove any crude matter. The samples were thoroughly washed with tap water and after that rinsed with distilled water to remove surface contaminants like soil and dust particles. The samples were dried using drying oven until a constant weight was achieved. The dried seed sample was then ground using a blender in the laboratory and sifted through a 0.457 mm sieve to remove large particles. The powdered samples were stored in an air tight container until needed. A 0.5 g of sample was weighed by using electronic balance (modelESJ200-4) with +0.0001 g; the weighed samples were then placed in digestion flask to await digestion.

2.5 Digestion of Brasicanigra seed Samples

Wet digestion of *Brasicanigra* for digestion purpose, 0.5 g of powdered and homogenized samples were weighed and transferred into a 100 mL round bottom flask. To this, 2 mL concentrated HNO_3 (69-72%), 1 mL of $HClO_4$ (70%) and 0.5 mL of H_2O_2 (30 %) were added. The mixture was then digested on Kjeldahl digestion apparatus (Gallenkamp, England) fitting the flask to a reflux condenser by setting the temperature at 120C^o for 30 min followed by 210C^o for 120 min until a clear solution was obtained following the optimized digestion procedure. After a total of 2:30 h, the digested solutions were allowed to cool for 30 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, 5 mL portions of deionized water were added and gently swirled to reduce dissolution of the filter paper by digest residue. The cooled digested samples were filtered into a 50 mL standard volumetric flask with a Whatman filter paper (110 mm) to remove any suspended or turbid matter. Subsequent rinsing of the filtrate with 5 mL deionized water was followed until the volume reached the mark. At this point, the solution was clear and colourless [10]. To each sample 1% „matrix modifier" lanthanum nitrate hydrate were added so that lanthanum may bind the phosphate and liberate calcium and magnesium in case large phosphate exist in the sample. For each *Brasicanigra* samples, triplicate digestions were carried out. Blank solutions were also digested accordingly in triplicate. The digested and diluted samples were kept in the refrigerator till the level of all the metals in the samples solutions were determined by FAAS.

2.6 Method validation for metal determination

In order to validate the analytical method, method validation parameters such as instrumental detection limit, limit of detection, limit of quantification, precision and accuracy studies were carried out[11,12].

2.6.1 Instrumental detection limit

Instrumental detection limit (IDL) is the smallest signal above background noise that an instrument can detect reliably. The IDL is calculated to be the concentration equal to three times the standard deviation of the reagent blank signal [11]. In this study, IDL for each metal was determined from analysis of ten calibration blanks and the concentration was calculated as:

IDL = 3 × sbl, where sbl is standard deviation of the calibration blank.

2.6.2. Limit of detection

Limit of detection (LOD) is the minimum concentration of analyte that can be detected but not necessarily quantified with an acceptable uncertainty. LOD for each metal was determined from analysis of blanks which were digested in the same digestion procedure as the actual samples. LOD was calculated as [11]:

$LOD = 3 \times S_{bl,}$ where S_{bl} is the standard deviation of the method blank.

2.6.3. Limit of quantification

The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample which can be quantitatively determined with acceptable uncertainty. LOQ was obtained from triplicate analysis of blanks which were digested in the same digestion procedure as the actual samples. The LOQ was calculated as [11]:

$LOQ = 10 \times S_{bl}$

where S_{bl} is the standard deviation of the method blank.

2.6.4 Precision

Precision was expressed as relative standard deviation (RSD) of replicate results. The relative standard deviations of the sample were obtained as:

%RSD = [(standarddeviation)/meanvalue] ×100

2.6.5. Recovery test

The accuracy of the analytical procedure was investigated by spiking a suitable known amount of the analyte metals into a test portion of the sample having a known concentration of the analyte, and by analyzing the spiked test portion along with the original sample. The recovery test for all samples was performed in triplicates. Recovery was then calculated as Recovery test [13]:

% R = [(Amount after spike – Amount before spike)/ Amount added] x100.

Instrument operating conditions

The flame atomic absorption spectrophotometer working conditions are as shown in Table 1.

Table1: Instrumental operating conditions for determination of metals in Brasicanigra seed sample using flame atomic absorption spectrophotometer

Element	Wavelength (nm)	Detection limi (mg/L)	Slit width (nm)	Lamp curren (mA) K
Ca	422.7	0.05	0.7	2.0
Mg	285.2	0.001	0.7	1.
Zn	213.9	0.005	0.7	2.0
Fe	248.3	0.05	0.2	3.5
Pb	217	0.04	0.7	3.0
Cu	324.7	0.02	0.7	1.5

2.7. Statistical analysis of data

Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) to assess significant variation in the mean concentrations of metals in *Brasicanigra* samples. A probability level of $p < 0.05$ was considered statistically significant. Pearson correlation coefficient was used to relate the levels of essential and non-essential metals between *Brasicanigra* samples. All statistical analyses were done by SPSS version 16.0 software for windows.

3. DISCUSSION

3.1. Instrument Calibration

The qualities of results obtained for metal analysis using FAAS are seriously affected by the calibration and standard solution preparation procedures. The instrument was calibrated using three series of working standards. The working standard solutions of each metal were prepared daily by diluting the intermediated standard solutions. The correlation coefficients of the metals were determined using prepared standards versus their corresponding absorbance. As shown in Table 2, the correlation coefficients of metals were found to be from 0.997-0.999, which confirmed good linearity of the signal with the concentration within the selected analytical range.

Table 2: Working standard concentration, correlation coefficient and equation of the calibration curves for determination of metals using FAAS

Metals	Concentration (mg/L)	Correlation values(r)	Regression equation(A*=mc+b)
Ca	0.50, 1.00, 1.50	0.999	A=0.033c

Mg	1.00, 2.00, 3.00	0.999	A=0.605c
Cu	0.50, 1.00,1.50	0.999	A=0.004+0.003
Fe	0.50, 1.00,1.50	0.997	A=0.055c+0.028
Zn	0.10,0.20,0.30	0.999	A=0.115c+0.0013
Pb	1.25,2.50,5.00	0.997	A=0.021c+0.015

* A = Absorbance, C = Concentration in mg/L

3.2. Evaluation of Method Validation

Method Detection Limit

As can be seen from Table 2, the method detection limit (MDL) of each element is above the instrument detection limit (IDL), indicating good sensitivity of the measuring instrument for analysis. The result shows both the MDL and MQL values were greater than the IDL; hence, the results of the analysis could be reliable.

Recovery Test

To ensure the reliability of the result obtained for the determination of the studied metals in the *Brasicanigra* samples, a recovery test was conducted. The percentage recoveries (%R) of the detected metals in the spiked *Brasicanigra* samples were calculated to be in the range 85% and 102.5%. This implies that, the measured results are within the acceptable range of 75 to 110%[15]. Therefore, excellent recovery results confirmed the suitability of the analytical method for the determination of metals in *Brasicanigra* samples. Then the percentage recoveries of the analytes were as shown in Table 3.

Table 4.Method detection limit, quantization limit (n=3, DLM=3S_{bl}, MQL=10S_{bl} in mg/100gand % recovery) for all metals determined in Brasicanigra samples.

Metals	MDL (mg/100g)	MQL (mg/100g)	% Recovery
Ca	0.006	0.02	102.5
Mg	0.003	0.01	93.5
Fe	0.09	0.3	85
Zn	0.003	0.01	95

Cu	0.054	0.18	98
Pb	0.12	0.4	97.5

Precision

For this study the precision of the results were evaluated by the standard deviation, and relative standard deviation (RSD) of the results of three replicate measurements (n = 3). The RSD values obtained for *Brasicanigra* samples ranged from 1.4% to 6.63% (Table 4), which ~~was~~ is under the required control limits ≤15% [16]. These results indicate that the proposed method was precise. The percent% relative standard deviation (RSD) of each metal is found in Table 4.

Table 4: percent (%) relative standard deviation (RSD) of each metal

Metals	Dejein		Awabel		Gozamen	
	Mean ± sd	RSD%	Mean ± sd	RSD%	Mean ± sd	RSD%
Ca	22.1±1.08	4.88	27.16±0.927	3.41	23.82±0.642	2.69
Mg	18.71±3.48	1.85	15.37±2.49	1.62	13.74±0.912	6.63
Fe	9.267±1.814	1.95	9.267±1.814	1.95	8.80±2.078	2.36
Zn	3.333±0.132	3.96	3.390±0.104	3.06	3.243±0.132	4.07
Cu	2.058±0.029	1.4	1.925±0.05	2.59	2.066±0.038	1.8
Pb	0.181±0.054	2.98	0.103±0.027	2.62	0.087±0.027	3.1

The Levels of the Essential and Non-essential Metals in the Brassica Nigra Seed Samples

The concentrations of essential and non-essential metals (Ca, Mg, Cu, Zn, Fe and Pb) in the samples were determined by FAAS using an air/acetylene flame at the wavelengths specific for each metal. All the analyses were carried out in triplicate. The summary of mean concentrations (mean ± SD) for the metals in *Brasicanigra* seed samples are presented in Table 5.

Table 5: Mean levels of essential and non-essential metals (mg/100 g) in the sample from the study areas (n=3)

Metals	Dejen	Awabele	Gozamen
	Mean ± sd	Mean ± sd	Mean ± sd

Ca	22.1 ± 1.08	27.16 ± 0.927	23.82 ± 0.642
Mg	18.71 ± 3.48	15.37 ± 2.49	13.74 ± 0.912
Fe	9.2667 ± 1.814	9.2667 ± 1.814	8.80 ± 2.078
Zn	3.333 ± 0.132	3.390 ± 0.1039	3.243 ± 0.132
Cu	2.058 ± 0.0288	1.925 ± 0.05	2.066 ± 0.038
Pb	0.181 ± 0.054	0.103 ± 0.027	0.087 ± 0.027

Brassica nigra contains higher amount of Ca followed by Mg and Fe. As can be seen from Table 5, there is relatively high concentration of Ca across the three geographical locations when compared with other metals studied. The concentration of Ca was 22.1± 1.08 mg/100 g to 27.16 ± 0.927mg/100g. The highest concentration of Ca (27.16mg/100g) was observed in Dejen district while the minimum 22.1± 1.08mg/100g was seen in Gozamin. Magnesium was the second element which was found in a relatively higher concentration across the sample areas. As it can be seen from the above Table, the amount of Mg across the sample areas ranges from13.74 mg/100g to 18.71mg/100g. The relatively higher concentration of Ca and Mg might be 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 [17]. If the soil used for cultivating the plant is highly fertilized with manure and organic residues, there is higher availability of K, Ca and Mg [17]. Due to this reason it may found in higher amount in *Brassica nigra* seed. Hence the plant has high amount of these elements.

Table5 shows that iron content ranging from 8.80 to 9.2667 mg/100 g. In the result, iron content was highest in Dejen and Awabel (9.26671mg/100g), while it was found lowest in Gozamin (8.80 mg/100g). The mean levels of Fe obtained upon analysis of the samples were also compared with safe limit recommended by FAO and WHO [18].

The acceptable limit for human consumption of zinc is 7.33 mg/100 g [18]. In this study, the concentration of zinc was found to be slightly higher in Awabel (3.39 mg/100 g) than the amount observed in Dejen (3.33mg/100g) and Gozamen (3.243 mg/100 g). The level of zinc ranges from 3.243 to 3.39 mg/100 g, which highly falls below the range of the recommended by FAO/WHO (i.e.7.33mg/100g) [18].

As shown in table 6, the level of copper in Dejen and Gozamin were found to be almost similar, i.e. 2.058 and 2.066mg/100 respectively while its concentration in Awabel was slightly lower (1.925mg/100g). The acceptable limit for human consumption of copper is 9.94mg/100 g [18]. The present investigation reveals that the level of copper varies from 1.925 to 2.066 mg/100 g, which falls below the safe limits for human health. This might be due to either the intake of this element by the plant may be low or the bioavailability of the element is very small in the plant [19,20].

The lead content in this study varies from 0.087 to 0.181 mg/100 g, which exceeds the safe limit (0.03mg/100 g) for human consumption set by FAO/WHO [18]. As shown in table 6Pb level, the amount of Pb obtained from Dejin is slightly higher than both from that of Awabel and Gozamen. Awabel is again slightly higher than that of Gozamen. The variation for Pb content in the *Brasicanigra* seed by sample site may be attributed to agricultural inputs such as fertilizers herbicides and insecticides containing Pb as an ingredient. Exposure to contamination during storage and transportation by cultivators could be other causes for the higher values [21].

Pearson correlation analysis of metals within Brassica nigra seed samples

In this particular study, to correlate the effect of the concentration of one metal over the other metal, the Pearson correlation (2-tailed) at 0.05 level of significance was employed. The relations for the *Brasicanigra* samples are shown in Table 6.

Table ***6: Pearson's correlation for Brasicanigra seed samples***

	Mg	Zn	Cu	Pb	Ca	Fe
Mg	1					
Zn	.035	1				
Cu	.159	-.551	1			
Pb	.29	-.095	.181	1		
Ca	-.285	.332	-.771	-.583	1	
Fe	.526	.437	.062	-.385	.182	1

Different authors suggest different interpretations of correlation coefficients. However, the following range has been suggested,± 0.10 to ± 0.29 small, ± 0.0.3 to ± 0.49 medium, ± 0.5 to ±1 large.

Accordingly, the values of Pearson correlation coefficient in Table 6 revealed that there is weak and/or moderate positive correlation between metals with each other except for some metals. The weak correlation indicates 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 large positive correlation of Fe with Mg in seeds and large negative correlation between Cu with Zn, Cu with Ca, and Ca with pb. Medium correlations were also found between some metals. For example, the table shows positive medium correlation between Ca with Zn, Fe with Zn and negative medium correlation between Fe with Pb.

As the table depicts, the correlation between many of the metals were small. For example, small positive correlations were found between Cu with Mg, Fe with Cu, Fe with Ca, Pb with Mg and Zn with Mg. On the other hand small negative correlations were observed between Pb with Zn and Ca with Mg. These correlations might be due to different size of seeds of the species, soil type, environmental conditions and capacity of the plant to accumulate specific element [23].

3.3. Statistical Analysis

One-way analysis of variance (ANOVA) was made at 95% confidence level. Comparing the means of all the three studied areas for their metal contents, at the 95 % confident level, the means are significantly different ($p < 0.05$) only for two metals. As the table shows the first metal on which significant difference observed was Ca with a p value of .001 which is below the set significant value ($p < 0.05$). The second metal on which significant difference observed was Cu with p value of .008 which is below the set significant value ($p < 0.05$). As depicted inn the above table, significant difference was not observed among other metals, i.e, Mg, Zn, Fe and Pb.

Table 7: Analysis of variance (ANOVA) between and within Brasicanigra sample at 95% confidence level

		Sum of Square	Df	Mean Square	F	Sig.
Mg	Between Groups	38.525	2	19.262	3.012	.124
	Within Groups	38.371	6	6.395		
	Total	76.896	8			
Ca	Between Groups	39.774	2	19.887	24.455	.001
	Within Groups	4.879	6	.813		
	Total	44.653	8			
Zn	Between Groups	.033	2	.016	1.078	.398
	Within Groups	.091	6	.015		
	Total	.124	8			
Fe	Between Groups	.436	2	.218	.060	.942
	Within Groups	21.813	6	3.636		
	Total	22.249	8			
Cu	Between Groups	.038	2	.019	11.870	.008
	Within Groups	.010	6	.002		
	Total	.048	8			
Pb	Between Groups	.015	2	.008	5.151	.050
	Within Groups	.009	6	.001		
	Total	.024	8			

Although Table 7 above shows significant differences on two metals, it does not show on which samples the differences were observed. Therefore another follow up analysis which is called a Post Hock analysis was conducted. The following table shows the post Hock analysis of the two metals on which significant differences were observed (Ca and Cu).

Table 8: Post Hock (Scheffe) Analysis of Metals on which Significant Differences wer Observed

Depenen Variable	(I) place	(J) place	Mean Difference (I-J	Std. Error	Sig.	95% Confiden Interval	
						Lower Bound	Upper Bound
Ca	Dejen	Awabel	-5.06333*	.73630	.001	-7.4248	-2.7018
		Gozamin	-1.72000*	.73630	.044	-4.0815	.6415
	Awabel	Dejen	5.06333*	.73630	.001	2.7018	7.4248
		Gozamin	3.34333*	.73630	.011	.9818	5.7048
	Gozamin	Dejen	1.72000*	.73630	.044	-.6415	4.0815
		Awabel	-3.34333*	.73630	.011	-5.7048	-.9818
Cu	Dejen	Awabel	.13333*	.03263	.018	.0287	.2380
		Gozamin	-.00833	.03263	.968	-.1130	.0963
	Awabel	Dejen	-.13333*	.03263	.018	-.2380	-.0287
		Gozamin	-.14167*	.03263	.014	-.2463	-.0370
	Gozamin	Dejen	.00833	.03263	.968	-.0963	.1130
		Awabel	.14167*	.03263	.014	.0370	.2463

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* mean difference is significant at the 0.05

The Post Hock analysis, Table 8 above, shows that there is significant difference between the samples of Dejen and Awable in Ca concentration with a p value of 0.001 as it is below the set significant level ($p < 0.05$). Similar significant differences were observed between the samples of Awabel and Gozamin in ca concentration with a p value of 0.011. In a similar way the analysis showed significant difference in the samples of Gozamin and Dejen with p value 0.044.

The second metal which showed significant difference across sample areas was Cu. And because of this, Post Hock analysis was conducted to see specifically on which sample areas cu concentration was observed. According to the above table, significant differences were observed between the sample areas of Dejen and Awable, on the one hand and Awabel and Gozamin on the other. The table indicated that there is significant difference between the samples of Dejen and Awabel in Cu concentration with p value of 0.018. The p value of Awabel and Gozamin is 0.014 which shows a significant difference in cu concentration of the samples taken. However, no significant difference was observed on the samples of Dejen and Gozamin in concentration of Cu since the observed p value was p=0.968 which is higher than the significant level set.

4. CONCLUSIONS AND RECOMMENDATIONS

The objective of this study was to determine the levels of selected essential and non-essential metals (Ca, Mg, Fe, Zn, Cu, and Pb) in black mustard seed (*Brasicanigra*). The levels of metals in three study areas of commercially available *Brasicanigra* were determined. The wet digestion method and the determination of selected metals in *Brasicanigra* by flame atomic absorption method were found to be efficient, precise and accurate. The efficiency of sample preparation and instrument were tested by assessing standard deviation and conducting recovery experiments. The content of metals in *Brasicanigra* samples was in the order of Camg/100 g> Mgmg/100 g> Femg/100 g> Zn mg/100 g> Cumg/100 g>Pbmg/100 g. Additionally the concentration of different metals detected in the three sampling sites was found as: Ca: Awabel>Dejein>Gozamen, Mg: Dejein>Awabel>Gozamen, Fe: Awabel and Dejein>Gozamen, Zn: Awabel>Dejein>Gozamen, Cu: Gozamen>Dejein>Awabele and Pb: Dejein>Awable>Gozamen. This observation helps to conclude that geographical location has an effect on the metal content of the seed.

Statistical analysis by using one way ANOVA also indicated that there is significant difference in mean concentration of metals under investigation. The ANOVA with post hock analysis showed that there are significant differences on Ca and Cu among Dejen, Awabel and Gozamin. This may be attributed to differences in soil composition, use of different fertilizers, pesticides, and may also be resulted from random and systematic errors in the experimental processes.

Based on the finding of this study, the following recommendations are forwarded. In order to aware users about the metal composition and to keep users safe from health risk, further study

should be carried out by collecting samples from all major *Brasicanigra* growing areas of the country. In this study the amount of lead was found in higher proportion. Therefore; further analysis of its content is recommended. Additionally, analysis of the soil metal content where *Brasicanigra* is growing and validating the method of analysis by characterizing using another instruments (ICP-MS, XRF) is very important.

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