



Determination of Selected Essential Nutrients and Toxic Heavy Metals In Lentil Seed harvested In North Shoa Zone Angollela and Tera Destrict

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Abstract

In this study the concentration of selected heavy metals(Pb ,Cu ,Cd ,Cr ,Hg, Fe, Zn) in lentil seeds harvested in Angewelega and Tera district(Chefanen and Chacha kebeles) have been evaluated using inductively coupled plasma optical emission spectrometer(ICP-OES). Samples were digested ready for analysis after a digestion of one hour by a mixture of HNO₃,HCl,HClO₄ and distilled water with hot plate digester. The recoveries for the metals were between 90 and 120% which revealed that an acceptable digestion method. The result showed that the amount of Copper, Lead, Chromium, Zinc and Iron were below the recommended values reported by WHO.On the other hand the concentration of Mercury and Cadmium were higher than the reported by WHO.

Keywords: lentil seeds, heavy metals, concentration and ICP-OES

1. Introduction

Lentil (*Lens Culinaris Medik.*) is the legume family annual plant having lens-shaped seeds. The plant is about 15 inches length and commonly two seeds grow in pods. Its stem is thin and normally herbaceous and weak;[1].Lentil being among the first domesticated crops and remain used as a source of food through successive farming for over 8000 years. Lentil is classified as drought resistant crop it can grow even in low annual rainfall distribution in the range of 280-300mm. And lentil can be well cultivated from cool temperature to subtropical dry zone environments. A soil pH from 6-8 is favorable for best production; however, it can also grow in a reasonable alkalinity. In Ethiopia, Lentil is mostly grown in the high lands. It provides reasonable source of proteins containing 22-35%, carbohydrates, fibers and minerals and thus play a vital role in developing countries in order to fulfill micronutrient deficiencies and alleviating malnutrition. When lentils are planted for the first time the in a field may need necessary nitrogen fixing bacteria [2].

Regarding to shape or size and color of seeds lentils are available in different types. The commonly used types of lentils used as food have red, brown and green colors. Compared to red and brown lentil Green colored are expensive due to their best and richest taste.

generally red lentils are preferable for soups and stews because of slighter sweet but brown colored lentils are cheap in cost and mild in flavor.

According to scholars estimation of world lentil production, the red types covers about 70% , green one takes 25% and the brown and other types covers only 5%.The green type mostly produce in USA and Canada, while the remaining of the world produces the red type lentils[3]. As a legumes plant,the contribution of lentil to the nitrogen in put on the farm by biological nitrogen fixation. The nitrogen fixation is very important especially in organic farming used by the following crop. In India, rice yields increased by 23.4% following lentil compared to wheat[4].

Lentil seed is used in numerous food foodstuffs used as a seed and in processed form [5]. Relatively Lentil seed contains high protein about 14 g per cooked cup (250 mL) similar to most legumes plants. Lentil is also rich in vitamins, minerals and proteins and the ideal to substitute peas and beans which are all high land crops. Lentil is a warm season crop with the optimum temperature range 27 °C to 30 °C for growth. Depending on the variety, it needs 90-120 days of frost free conditions from planting to maturity[7].

Minerals are important also for the metabolism and growth of plants and they have vital role in the biosynthesis of growth hormones[8].According to

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research reports supply of excess heavy metals can be affected the growth and metabolism of many plant species[9].heavy metals like Ni, Fe and Cr in lentil were more easily transported to shoots and roots and accumulated when compared to seeds and their presence in the fruits[10]. Here in this research focus on the analysis of heavy metals accumulation in lentil seeds harvested in north shoa zone, Angewelega and Tera district in Chacha, Chekana kebeles.Studies have revealed that refuse soils in dumpsite contain diverse types and concentrations of heavy metals (transition elements with higher atomic mass [11,12]. Currently, it has been reported that heavy metals accumulated in the soil and persists in soils at an environmentally hazardous levels [13].

2. Materials and Method

2.1. Materials

In this study, ICP-OES, conductivity meter, pH meter and air circulating oven were used. Distilled water, 72% HNO₃ or 70% HClO₄, HCl from (Research-lab fine chem industries mumbai, india) were used for the sample digestion.

2.2. Method

Samples of lentil seeds were collected from the farmers of Angewelega and Tera district in the field.The samples were first washed with water several times to remove dusts and dirties. Then the lentil seed samples were dried, crushed and homogenized in to fine powder using mortar and pestle then stored in plastic bag at for further experiments.

2.3. Procedures

5 g of lentil seeds flour were transferred to a clean and labeled crucible according to the samples. The process of Dry-ashing was performed by stepwise increase of the temperature up to 550 °C in muffle furnace and left it for 6 hrs. Then the samples removed from the furnace and allowed to cool. Concentrated HNO₃ (2.5ml) was added for every sample after the ash was wetted with water. The digestion had been carried out at a temperature of 95 °C for 1hr.Then it was dissolved by 9.25% in 5 mL HCl and again it was digested on a hot plate until the white smokes ceased to occur. Then 2 mL 70% HClO₄ was added to the cooled solution and heating again until clear solution was appeared. Eventually all HNO₃ disappeared through evaporation and the HClO₄ fumes appeared which consequently vaporized. The final residue was refluxed in the beaker after it was treated with 5 mL of concentrated HCl; then an equal volume of water was added with

Eth. J. Indig. Know. Appl. Sci.

subsequent evaporation. This refluxing process was repeated three times. Finally, the mixture was warmed temporarily after addition of 1.0 mL concentrated HCl ; then 15 mL of water was added and heated for 15 min. additional 20 mL of distilled water was added after cooling, and then filtration occurred using Whitman filter paper. The filtered samples were diluted to 50 mL then transferred to a 50 mL polyethylene bottle for investigation. A replicate samples were prepared for each element. ICP-OES analysis was made for every element and its standard solution.

2.4. Statistical (DATA) Analysis

Statistical data analyses were performed using the Microsoft EXCEL(version 2010).Analysis of test(P<0.05) were employed to study statistical significance of difference in the mean concentration of heavy metals between lentil seeds harvested in the two kebeles using SPSS program. All treatments in the experiment were organized and analyzed in convenient way to make meaningful and readable.

The relationship between heavy metals contents properties in the lentil seeds with heavy metals were analyzed by Pearson's correlation coefficient (bivariant method) for each lentil seeds sample. The correlation coefficient was close to +1 or -1. This indicated a good relation between the two variables and the correlation coefficient was close to zero, showing no relationship between the two variables at a significant level of 0.05 and 0.01. If $r > 0.7$, the variables strongly correlated whereas the moderate correlation showed r values between 0.5 and 0.7 of two different parameters[14].

Please look carefully at this statement as it needs critical revision. Positive and highly significant correlation ($r = 0.99^*$), ($r = 0.99^*$) between heavy metal lead with cadmium and iron with copper respectively for Chacha. Similarly there was positive significant correlation($r=0.99^*$) between heavy metal copper with zinc for Chefanen. However, the degree to which two securities wer negatively or positively correlated might vary over time and are almost never exactly correlated, all the time. This condition may happen sometimes because one physicochemical property may affect other and it may make to don't have consistent.

3.5. Method Validation

Method validation was applied to evaluate the reliability and reproducibility of analytical results. The typical validations characteristics such as precision,

accuracy, range, quantification limit, detection limit and linearity were considered in the study.

Table 1. Operating condition of ICP-OES instrument for determination of heavy metals.

Heavy metals	Wave Length (nm)	IDL (mg/L)	Range (mg/L)	Lamp Current (mA)	Temperature (° C)	Plasma Power (W)	Main Argon Pressure (bar)	Instrument model
Lead(Pb)	220.353	0.0007	0.0007-1.68	607	51.53	1400	6.75	ARCOS12
Copper	324.75	0.006	0.006-3.36	607	51.53	1400	6.75	ARCOS12
Zinc	213.856	0.008	0.008-3.36	607	51.53	1400	6.75	ARCOS12
Mercury	184.950	0.003	0.003-3.84	607	51.53	1400	6.75	ARCOS12
Cadmium	214.438	0.0001	0.0001-1.68	607	51.53	1400	6.75	ARCOS12
Iron	259.941	0.0018	0.0018-4.8	607	51.53	1400	6.75	ARCOS12
Chromium	267.71	0.001	0.001-1.68	607	51.53	1400	6.75	ARCOS12

3. Results and Discussion

Series concentrations prepared by serial dilution of the metals and correlation coefficient of the calibration curves were shown in table-1.

The concentration of Pb, Zn, Hg, Cu, Fe, Cr, Cd in each test agreed with each other as shown in the table 2 below. The precision of the measurements is good for each metal as indicated by low value of standard deviations. This indicated that a good precision since every measurement for each metal close to the average value. Moreover, the precision of zinc and iron was highest compared with the other heavy metal determined.

Table 3 and 2 show the concentration of Pb, Zn, Hg, Cu, Fe, Cr, Cd in each trial agrees with each other. The lower standard deviations value also indicates that the good precision.

Heavy metals	Concentration Standards(mg/L)	Correlation coefficient (R ²) of calibration curves
Lead(Pb)	0,0.028,0.056,0.086, 0.280.56,0.84,1.12,1.4	0.990
Zinc(Zn)	0,0.056,0.112,0.168, 0.5,1.12,1.68,2.24,2.8	0.998
Cadmium (Cd)	0,0.028,0.056,0.084 0.28,0.56,0.84,1.12, 1.4	0.999
Mercury(Hg)	0,0.064,0.128,0.192, 0.64,1.28,1.92,2.56, 3.2	0.999
Copper(Cu)	0,0.056,0.112,0.168, 0.56 1.12,1.68,2.24,2.8	0.999
Iron(Fe)	0,0.056,0.112,0.168, 0.56,1.12,1.68,2.24, 2.8	0.999
Chromium(Cr)	0,0.028,0.056,0.084 ,0.28,0.56,0.84,1.12, 1.4	0.999

Table 3.1. Series of working standard and correlation coefficient of the calibration curves

Table 3.2. The concentration of heavy metals in lentil seeds determined by ICP-OES.

Metals	Study area	
	Chacha kebele	Chefanen kebele
Pb mg/kg	1.1348±0.2952	1.009± 0.2246
Zn mg/kg	4.5793±2.2611	4.439± 3.0499
Cd mg/kg	0.1515±0.0311	0.1367±0.0369
Hg mg/kg	0.5945±0.0440	0.7493±0.2896
Cu mg/kg	0.7615±0.3763	0.662± 0.4809
Fe mg/kg	4.8313±3.4866	2.622± 1.9242
Cr mg/kg	0.2882±0.1197	0.295± 0.1600

Table 3.3. Comparison of heavy metals with the recommended concentration by WHO/FAO in lentil seeds.

Heavy metals	Conc.(mg/kg) at Chacha kebele	Conc.(mg/kg) at Chefanen kebele	Reported by WHO in mg/kg
Lead(Pb)	1.1348	1.009	1.5
Zinc(Zn)	4.5793	4.439	100
Cadmium(Cd)	0.1515	0.1367	0.1
Mercury(Hg)	0.5945	0.7493	0.1
Copper(Cu)	0.7613	0.662	10
Iron(Fe)	4.8313	2.622	425
Chromium(Cr)	0.2882	0.295	1.3

Lead

Table 3.1 shows that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of lead in lentil seed was 1.1348 mg/kg ±0.2952 and 1.009± 0.2246 in chacha and Chefanen kebele respectively. The permissible limit according to WHO/FAO is 1.5 mg/kg [15]. The amount of lead in both samples was slightly lowered compared with lead standard in WHO/FAO.

Zinc

As shown in the table 3, the amount of Zinc in lentil seed were 4.5793 mg/kg ± 2.2611 and 4.439 mg/kg ±3.0499 in chacha and Chefanen kebele respectively. The permissible limit is 100mg/kg[15].The concentration of zinc in the samples were very much lower when compared with the standard indicated by WHO/FAO.

Cadmium

The Cadmium concentration in lentil seed cultivated in chacha and Chefanen kebeles were 0.1515 mg/kg ± 0.0311 and 0.1367 mg/kg ± 0.0369. The acceptable limit according to WHO/FAO is 0.1 mg/kg [15].The result indicating that the concentration cadmium was above WHO/FAO limit. Inhalation of Cd fumes can cause acute pulmonary and kidney damage .It also cause severe and long-lasting poisoning causing adverse effects on vascular and immune system [16]. The waste material and the fertilizers might increase the concentration of cadmium in lentil seed as observed in this study.

Mercury

As indicated on table 3, the concentration of mercury in lentil seed in chacha and Chefanen kebeles were

0.5945 mg/kg $\pm$ 0.0440 and 0.7493 mg/kg $\pm$ 0.2896 respectively. According to WHO/FAO, the acceptable concentration limit of Mercury is 0.1 mg/kg. Thus, the mercury concentrations in the samples were excessed compared with standard. The waste material and the fertilizers might increase the concentration of mercury in lentil seed cultivated in this area.

Copper

Copper is a micro element essential for plant growth and available in the soil. The amount of Cu in lentil seed in Chacha and Chefanen kebele were 0.7615 mg/kg $\pm$ 0.3763 and 0.662 mg/kg $\pm$ 0.4809 respectively. According to WHO (1996) standard the acceptable concentration limit is around 10 mg/L. The amount of Cu in the sample was below lowered standard (10mg/L) value. Copper content varies because of the soil type and pollution sources in the surrounding environment[17]. Therefore the low concentration of copper indicated that the absence of contaminant sources in the two kebeles.

Iron

The concentration of iron found in lentil seed in chacha and Chefanen kebeles very low (4.8313mg/kg $\pm$ 3.4866 and 2.622mg/kg $\pm$ 1.9242 respectively) when compared to WHO limit which is 425mg/kg [15].

Chromium

As indicated in the table 3 above, concentration of chromium in lentil seed in Chacha and Chefanen kebele were 0.2882mg/kg $\pm$ 0.1197 and 0.295mg/kg $\pm$ 0.1600 respectively. however, the recommended concentration limit of chromium in lentil seed by WHO standard is 1.3 mg/L $\pm$ 0.0202[15]. Since the Cr is not essential for plant growth, its uptake by plant is generally low even it may not detected in some plant unless the environment contaminated from different Cr containing discharge to the agricultural land and the surroundings[18].

In general, the amount heavy metals (Pb, Zn, Cd, Cu and Fe) in lentil seed cultivated in chacha kebele was greater than in Chefanen kebele. On the other hand the concentration of Hg and Cr are higher in Chefanen kebele.

3.1. Recovery Test

The efficiency of digestion method was evaluated by addition of known amount of each metal in a 5g of sample. For recovery analysis, 4mg/kg of each heavy metal was spiked to the sample. And the percent recovery of each metal was calculated by the formula indicated below.

$$\text{Percentage recovery} = \frac{\text{Conc. spiked} - \text{Conc.unspiked sample}}{\text{Amount spiked}} \times 100$$

Table 3.1.1. Percentage recovery of the sample at Chacha kebele

Heavy metals	Unspiked Amount(mg/kg)	Amount added in(mg /kg)	Amount after spiked(mg/kg)	%Recovery $\pm$ SD
Lead(Pb)	1.1348	4	5.759	115.6 $\pm$ 0.2952
Zinc(Zn)	4.5793	>>	9.203	115.59 $\pm$ 2.2611
Cadmium(Cd)	0.1515	>>	4.870	117.96 $\pm$ 0.0311
Mercury(Hg)	0.5945	>>	5.0657	111.78 $\pm$ 0.0440
Copper(Cu)	0.7615	>>	5.600	120.96 $\pm$ 0.3763
Iron(Fe)	4.8313	>>	9.0247	104.83 $\pm$ 3.4866
Chromium(Cr)	0.2882	>>	4.0096	93.03 $\pm$ 0.1197

Table 3.1.1 and table 3.1.2 showed that the percent recovery of the samples collected from both Chacha kebele and Chefanen kebele. Thus, The percentage recovery values of Zn, Hg, Pb, Cu, Fe and Cr were within the acceptable range of (80 to 120%) except for Cd(120.4%) from Chefanen kebele. Generally, the percent recovery results indicated that good accuracy for the analysis procedure[19]. Therefore, the result confirmed that the reliability of the method.

Table 3.1.2. Percentage recovery of the sample at Chefanen kebele

Heavy metals	Unspiked (mg/kg)	Amount added in(mg/kg)	after spiked (mg/kg)	%Recovery $\pm$ SD
Lead(Pb)	1.009	4	4.685	91.9 $\pm$ 0.2246
Zinc(Zn)	4.439	>>	9.010	114.275 $\pm$ 3.0479
Cadmium (Cd)	0.1367	>>	4.9538	120.42 $\pm$ 0.0369
Mercury(Hg)	0.7493	>>	5.091	108.54 $\pm$ 0.2896
Copper(Cu)	0.662	>>	5.4468	119.62 $\pm$ 0.4809
Iron(Fe)	2.622	>>	7.333	117.77 $\pm$ 1.9242
Chromium(Cr)	0.295	>>	5.0154	118.01 $\pm$ 0.1600

3.2. Method detection limit (MDL) and Method quantification limit(MQL)

The two important parameter in method validation are detection limit and quantification limit. Detection limit is the lowest concentration of analyte that can be detected by the applied method and stated with 95% confidence that the concentration of the analyte is greater than zero, for the present study, the detection limit was found by three times the standard deviation of the mean reagent blank signal. Analysis of blank of all metals of interest were performed and the standard deviation of the blank reagents was calculated according to reference number [20].

Method quantification limit is the lowest analytical concentration that could be quantitatively measured in sample matrix with stated accuracy and precision; it is similar to the concentration that gives a signal ten times the standard deviation of blank. Seven blank samples were digested following the same procedure as for the sample was digested and the concentration of each elements determined by ICP-OES.

Method detection limit of each element is calculated as three times the standard deviation of blank

Detection limit= $3 \times$ Standard deviation of blank

Quantification limit= $10 \times$ Standard deviation of blank

Table 3.2.1. Detection and quantification limit of heavy metals in lentil seed samples..

Heavy metals	MDL in mg/kg $\pm$ SD	MQL in mg/kg $\pm$ SD
Lead(Pb)	0.036	0.12
Zinc(Zn)	0.012	0.04
Cadmium(Cd)	0.003	0.01
Mercury(Hg)	0.003	0.01
Copper(Cu)	0.003	0.01
Iron(Fe)	0.015	0.05
Chromium(Cr)	0.012	0.04

Table 3.2.1 showed that the experimental analysis of MDL, and MQL. As shown in the table, the MDL was higher than the detection limit of the instrument (ICP-OES), which confirmed that the method was acceptable and the heavy metals concentration in the sample were better detected by the instrument.

The MQL was the least which showed that the accuracy is good. Thus, the method (procedure) was more reliable.

4. Conclusion

In the current study, the heavy metals concentrations was examined by inductively coupled plasma optical emission spectrometer (ICP-OES). The concentrations of Cu, Pb, Cr, Zn and Fe were lower than the standard limit reported by WHO. However, the Hg and Cd concentration were greater than the reported values by WHO. Thus, the concentration of Hg and Cd might cause health problems. Inhalation of Cd fumes can result acute pulmonary and kidney damage and also it may cause chronic poisoning causing adverse effects on vascular-system and immune [16]. The excess Hg concentration in lentil seed might result from household material like cosmetics containers, inorganic and organic Hg containing compounds that entered to the soil and water which was lentil seed plant could grow.

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