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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 Angewelela and Tera district(Chefanen and Chacha kebeles) have been analyzed by 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 concentrations of Copper, Lead, Chromium, Zinc and Iron were lower than the recommended values reported by WHO. On the other hand the concentration of Mercury and Cadmium was higher than the values reported by WHO

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

1. Introduction

Lentil (Lens Culinaris Medik.) is a brushy annual plant of the legume family, grown for its lens-shaped seeds. It is about 15 inches tall and the seeds grow in pods, usually with two seeds in each its stem is thin, square and generally herbaceous and weak;[1].Lentil being one of the first crops to be domesticated by man and continue to be an important food source for over 8000 years through subsequent cultivation. Lentil is considered as drought resistant crop that can tolerate low annual rainfall distribution even in the range of 280-300mm in regard to temperature ,lentil can grow in different environments from cool temperature to subtropical dry zone.A soil pH of 6-8 is conducive for lentil production ,but it can also tolerate a moderate alkalinity. Lentil is mainly grown in the high lands of Ethiopia where rainfall is usually high. It provides affordable source of dietary proteins (22-35%), minerals, fibres, and carbohydrates to people and play a vital role in alleviating malnutrition and micronutrient deficiencies in developing countries. When lentils are planted in a field for the first time the necessary nitrogen fixing bacteria must be provide [2].

There are many different types of lentils, concerning seed color, shape or size. The most common types used in cooking are brown, red and green lentils. Brown lentils are mild in flavor and the least expensive generally red lentils have slighter sweeter taste than brown ones and are better for soups and stews. Green lentil are the finest and richest tasting but most expensive.

Some scholars estimated that about 7 0% of the world lentil production was the red types,25% green type and 5% brown and other types. Canada and USA mainly produce the green type, whereas the rest of the world produces the red type lentils[3]. As a kind of legumes plant crops, lentil contribute to the nitrogen in put on the farm due to the biological nitrogen fixation. The nitrogen fixed by lentils may be used by the following crop which is important especially in organic farming. There was a 23.4% increase in rice yields following lentil compared to wheat in India[4].

Lentil is used in several food products, both as a whole seed and in processed form [5]. Like most legumes plant Lentil seed contains relatively high protein about 14 g of protein per cooked cup (250 mL).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 requiring 90-120 days of frost free conditions from planting to maturity (depending on the variety). The optimum temperature range for growth is between 27 ^{0}C and 30 ^{0}C. Lentil is considered to be heat and drought tolerant[7].
Metals are also essential for the normal growth and metabolism of plants and play very important role in the biosynthesis of some enzymes and growth hormones[8].The growth and metabolism of many plant species were reported to be affected adversely by excess supply of heavy metals[9].The accumulation of heavy metals in lentil grown on metalliferous mine

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spoil also found that Cr, Ni and Fe were more easily transported to shoots and roots as compared to seeds and their presence in traces in the fruits[10]. Here in this experimental research attempts will take to study the accumulation of heavy metals in seeds of lentil grown in north shoa zone,Angewelela and Tera district two different kebele(Chacha, Chefana). Heavy metals are elements of high molecular masses, most of which belong to the transition elements[11].Studies have shown that soils of refuse dumpsite contain different kinds and concentrations of heavy metals[12]. In recent times, it has been reported that these elements accumulate and persist in soils at an environmentally hazardous levels [13].

2. Materials and Method

2.1. Materials

In this study, pH meter, conductivity meter, ICP-OES, air circulating oven and digestion tubes were used. Distilled water, 69% to 72% HNO_3 or 70% $HClO_4$, HCl (Research-lab fine chem industries mumbai, india) were used for the digestion purpose.

2.2. Method

Raw lentil seeds samples were collected from the agricultural lands of Angewelela and Tera district from the field after crop harvested.The lentil seeds samples were first washed with distilled water several times to remove dusts and dirty particles. And then the dried lentil sample were crushed and homogenized in to fine powder using porcelain mortar and pestle store in plastic bag (zip lock) at room temperature for further experimental use.

2.3. Procedures

5 g of lentil seeds flour were weighed and transferred to a clean crucible, which were labelled according to the samples and dry-ashing process would be carried out in muffle furnace by stepwise increase of the temperature up to 550 ^{0}C and left to ash at this temperature for 6 hrs. The samples were removed from the furnace and allowed to cool. The ash was wetted with water and 2.5 mL of concentrated HNO_3 was added for each sample. The digestion had been performed at a temperature of 90 to 95 ^{0}C for 1hr.The ash had been dissolved in 5 mL of 9.25% HCl and digested again on a hot plate until the white fumes ceased to exist and samples reached to 2 mL. Then 2 mL 70% $HClO_4$ was added to the cooled solution and heating resumed until clear solution were appeared. As all HNO_3 eventually evaporated, fumes of $HClO_4$ appeared which then removed by evaporation. The residue had been treated with 5 mL of concentrated HCl and the acid was refluxed in the beaker; an equal volume of water was then added with subsequent evaporation to dryness. This refluxing process with concentrated HCl followed by evaporation to dryness repeated. Finally, 1.0 mL concentrated HCl added and the mixture was warmed briefly; then 15 mL of water was added and the solution has been heated for about 15 min. After it was cooled 20 mL of distilled water were added and filtered using Whitman filter. The filtered samples were diluted up to the mark of 50 mL standard volumetric flask, and transferred to a 50 mL polyethylene storage bottle until analysis. A triplicate samples were prepared for each element. ICP-OES measurement was then made for each individual element along with the appropriate standard solution.

2.4. Statistical (DATA) Analysis

All statistical analyses were performed on hp pc computer using the Microsoft EXCEL(version 2010).Analysis of test($P<0.05$) were employed to examine statistical significance of difference in the mean concentration of heavy metals between lentil seed using statistical package for social sciences(SPSS) program. All treatments in the experiment were organized and analyzed in convenient way to make meaningful and readable.

Correlation analysis is a bivariant method which was applied to describe the relation between two different parameters. The relationship between contents of heavy metals properties in the lentil seeds with heavy metals were analyzed by Pearson’s correlation coefficient for each lentil seeds sample. The high correlation coefficient (near +1 or −1) means a good relation between two variables and correlation around zero means no relationship between them at a significant level of 0.05 and 0.01. Correlation can be also strongly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 show moderate correlation between two different parameters[14].

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 positive significant correlation($r=0.99^*$) between heavy metal copper with zinc for Chefanen. However, the degree to which two securities are 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

Validation was concerned with assuring that a measurement process produced valid measurements. Results from method validation were used to judge the quality, reliability and consistency of analytical results. It was an integral part of any good analytical practice. The following typical validations characteristics were considered in the study accuracy, precision, detection limit, quantification limit, linearity and range.

Table -1 Instrumental operating condition for determination of heavy metals using ICP-OES from lentil seeds.

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 of working standard concentration and correlation coefficient of the calibration curves were shown in table-1 for the determination of heavy metals in lentil seeds using ICP-OE

As shown in the table-2 below, the concentration of Pb, Zn, Hg, Cu, Fe, Cr, Cd in each trial agree with each other. The standard deviations for each metal were low which indicates the precision is high. This confirmed that the precision is good because each measurement in each metal close to the average of the series. However, the precision in zinc and iron was highest compared with the precisions in other heavy metal determined. Therefore the measurement in iron and zinc was the most precise.

Table -3.2 shows the concentration of Pb, Zn, Hg, Cu, Fe, Cr, Cd in each trial agree with each other. The standard deviations for each metal were low which indicates the precision is high. This confirmed that the precision is good because each measurement in each metal close to the average of the series. However, the precision in zinc and iron was highest compared with the precisions in other heavy metal determined.

Table 3.1 series of working standard and correlation coefficient of the calibration curves

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 3.2 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 showed 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 concentration of lead in both samples were slightly lowered compared with lead standard in WHO/FAO.

Zinc

As shown in the table 3,the concentration 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.Cadmium fumes inhalation can cause acute pulmonary and kidney damage .It may cause acute and chronic poisoning causing adverse effects on immune and vascular-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 wwre 0.5945 mg/kg ±0.0440 and 0.7493 mg/kg ±0.2896 respectively. According to WHO/FAO, the acceptable concentration limit of Mercury is 0.1 mg/kg. Thus, the concentrations of mercury 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 which is essential in plant growth and occurs generally in the soil. the copper concentration in lentil seed in Chacha and Chefanen kebele were 0.7615 mg/kg ±0.3763 and 0.662 mg/kg ±0.4809 respectively. According to WHO (1996) standard the acceptable concentration limit is around 10 mg/L. The concentration of copper in the sample was lowered compared with copper standard (10mg/L). Copper content has been reported to differ according to the soil type and pollution source [17]. Thus the low concentration of copper might be due to the soil type and the absence of contamination.

Iron

The concentration of iron found in lentil seed in chacha and Chefanen kebeles very low (4.8313mg/kg ±3.4866 and 2.622mg/kg ±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 ±0.1197 and 0.295mg/kg ±0.1600 respectively. however,the recommended concentration limit of chromium in lentil seed by WHO standard is 1.3 mg/L ±0.0202[15]. Chromium is not essential for plant growth, it was not detected in some plant sites due to the fact that uptake of chromium by plant is generally low [18].this might confirm the soil and the water were not contaminated by different discharge wastes.

Generally the concentration heavy metals such as lead, zinc, cadmium, copper and iron in lentil seed cultivated in chacha kebele greater than in Chefanen kebele. On the other hand the concentration of mercury and Chromium are higher in Chefanen kebele. Cadmium and mercury concentrations were higher than the recommended concentration by WHO/FAO.

3.1. Recovery Test

The efficiency of the digestion procedure was checked by adding known concentration 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.

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

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±SD
Lead(Pb)	1.1348	4	5.759	115.6±0.2952
Zinc(Zn)	4.5793	>>	9.203	115.59±2.2611
Cadmium(Cd)	0.1515	>>	4.870	117.96±0.0311
Mercury(Hg)	0.5945	>>	5.0657	111.78±0.0440
Copper(Cu)	0.7615	>>	5.600	120.96±0.3763
Iron(Fe)	4.8313	>>	9.0247	104.83±3.4866
Chromium(Cr)	0.2882	>>	4.0096	93.03±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±SD
Lead(Pb)	1.009	4	4.685	91.9±0.2246
Zinc(Zn)	4.439	>>	9.010	114.275 ±3.0479
Cadmium (Cd)	0.1367	>>	4.9538	120.42± 0.0369
Mercury(Hg)	0.7493	>>	5.091	108.54± 0.2896
Copper(Cu)	0.662	>>	5.4468	119.62± 0.4809
Iron(Fe)	2.622	>>	7.333	117.77± 1.9242
Chromium(Cr)	0.295	>>	5.0154	118.01± 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 confidently by the analytical method with a give certainty and stated with 95% confidence that the analyte concentration 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 [20]. Method quantification limit is the lowest concentration of analyte that can be measured in sample matrix at an acceptable level of precision and accuracy, it is the same as the concentration which 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× Standard deviation of blank

Quantification limit= 10 × 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 ±SD	MQL in mg/kg ±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 is greater than the detection limit of the instrument (ICP-OES), which confirmed that the method was acceptable and the instrument better detected the concentration of the heavy metals in the sample.

The MQL was the least which indicated that the accuracy is high. Therefore, the method (procedure) was more reliable.

Conclusion

In this study, the concentration of the heavy metals have been analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES). The concentrations of copper ,lead ,chromium, zinc and iron were lower than the values reported by WHO. The concentration of mercury and cadmium was higher than the values recommended by WHO. Thus, the concentration of mercury and cadmium might cause health problems since their concentration were above the recommended level (0.7493 mg/kg and 0.5945mg/kg for mercury and cadmium respectively) Cadmium fumes inhalation can cause acute pulmonary and kidney damage. It may cause acute and chronic poisoning causing adverse effects on immune and vascular-system [16].The excess mercury concentration in lentil seed might result from house hold material like cosmetics containers, inorganic and organic mercury compounds that entered to the soil and water which was lentil seed plant could grow.

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