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Investigation of heavy metal accumulation in mung bean (*Vigna radiate*) through inductively coupled plasma optical emission spectroscopy

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Although mung bean is cultivated for its nutritional crop in a different region of Ethiopia through irrigation, its production is hindered by excess accumulation of heavy metals in the environment. The presence of excess heavy metals in the environment subsequently leads to the contamination of consumable products, hampered crop productivity, and subsequently affects human health. The present study aimed to determine the concentrations of selected heavy metals (Pb, Cu, and Cr) in the mung bean collected from Shewarobit, Northcentral, Ethiopia, using inductively coupled plasma optical emission spectroscopy (ICP-OES). Dried seeds of mungbean were digested in a microwave oven by nitric acid and hydrogen peroxide for 1hr at a temperature of 90 °C to 95 °C by a hot plate digester and then the concentration of Pb, Cu, and Cr was measured through inductively coupled plasma optical emission spectrophotometry (ICP-OES). The result revealed that the concentration of heavy metals in the samples was 2.51 mg/L, 6.53 mg/L, and 0.38 mg/L for Pb, Cu, and Cr, respectively. Copper (Cu) was found to be higher, followed by lead (Pb) and chromium (Cr). The overall accuracy and applicability of the method were confirmed through spiking experiments (recovery) in the range of 80 to 120%, which account for 110.5% ± 0.039, 96.0% ± 0.236, 102.0% ± 0.085 for Pb, Cr, and Cu, respectively. The concentration of Cu and Cr in mung bean falls under the permissible limit. However, the amount of lead is higher than the permissible limit and it is unsafe to consume. Thus, appropriate measures should be done to adjust its concentration.

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Introduction

Mung bean is a leguminous annually pulsed crop that mostly grows in lowland areas of Ethiopia [1]. Because of its sensitivity to waterlogging, much of its production is dependent on irrigation [2]. It is used as a source of proteins, essential amino acids, vitamins, and minerals. It also contains thiamine, iron, magnesium, and other nutrients and is a good source of folate [3]. Although mung bean is cultivated as an important nutritional crop in different parts of Ethiopia, its production and nutritional composition are affected by different abiotic factors. These include the accumulation of excess heavy metals.

Heavy metals are present in all types of ecosystems. It exists naturally in soil, but anthropogenic activities such as agricultural, mining, and industrial activities could enhance their concentration in soil. The application of chemical fertilizers in agricultural soil can increase the level of heavy metals [1]. Waste from such activities contributes to the increment of heavy metal concentration which leads to the pollution of water bodies and soil, affecting the growth of plants and aquatic life [2].

Research attests that heavy metals are important for the productivity of plants [4]. However, the development and productivity of many plants/crops have been affected by the excess supply of heavy metals from the environment [5]. Accumulation of excess heavy metals alter the normal physiology of human and may result in serious health problems. For instance, intake of high concentrations of Cu ions may result in the irritation of the nose, mouth, and eyes as well as bringing about headaches, stomach aches, dizziness, vomiting, and diarrhea, with an increased risk of tubular dysfunction [6]. Pb ions may result in adverse kidney malfunction and high blood pressure in adults, whereas they may inhibit physical and mental development in children [7]. A large portion of these pollutants can ultimately be traced to various consumable items such as dietary supplements, natural medicine remedies, cosmetics, and various other commercial products [3].

Different analytical techniques are used for determining trace or ultra-trace concentrations of heavy metals in samples like plants [8]. Inductively coupled plasma/optical emission spectrometry (ICP/OES) is a spectroscopic technique appropriate to detect trace elements in given samples. This method was used to determine the concentration of As, Cu, Zn, Cd, and Pb in soft drink samples as mentioned elsewhere [9]. Notably, the research also detected Cr, Cd, Cu, Fe, Mg, Mn, Ni, cobalt (Co), Zn, and aluminum (Al) in four edible parts of the marine fish species [10]. The advantages of using ICP-OES over other elemental analysis techniques such as inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectrometry (AAS) include its wide linear dynamic range, high matrix tolerance, multi-elements capability, high sensitivity, good reproducibility, low matrix effect and wide dynamic linear range for rare earth elements (REEs) analysis and its needs for a short time [11]. This method has become a very thought-provoking technique to analyze major and trace elements in plant samples [12]. Appropriate sample treatment is imperative to obtain precise and accurate results [13]. Many ash procedures such as dry and wet ash procedures are known to be very slow and difficult to carry out. Microwave digestion is an eminent sample preparation method that is efficient and rapid [14].

Rapid industrialization and current agricultural practices may result in the accumulation of heavy metals in the environment [15]. The usage of different fertilizers and chemicals may increase the accumulation of heavy metals in the environment fertilizers [4, 16, 17]. These pollutants are prevailing in the environment for a longer period, as they are not easily degraded by soil microorganisms and therefore can easily be absorbed by plants [18].

The presence of heavy metals within the crop in higher concentration causes different environmental problems: To mention some; a) Poor germination and low productivity of the crop [19] which may alter its nutritional values [5], and b) serious environmental damages to the soil, cropping, vegetation, and in turn human health [20]. It also affects human health upon consumption [21].

Studies have shown that soils of dumpsites contain concentrations of heavy metals [18]. There is a large number of dumpsites in Shewarobit town due to urbanization and poor waste management system which may

result in the accumulation of heavy metals in the water used for irrigation [5, 22]. Besides, the farmers and producers of mung bean have applied different insecticides, fertilizers, and herbicides, which may pollute the soil and facilitate the uptake of excess heavy metals. Due to the metal toxicity and their adverse effects on public health and the environment, it is therefore essential to precisely measure their levels and take appropriate management. Notably, accurate monitoring of heavy metal concentrations in mungbean is crucial to minimize health hazards resulting from exposure to such toxic substances. To this end, the present study aimed to determine the concentrations of selected heavy metals in mung bean through inductively coupled plasma optical emission spectroscopy (ICP-OES).

Methods and materials

Description of the study area and sample collection

The study was conducted at the chemistry Department of Debre Birhan University, Ethiopia, and the Soil and Water Analysis Laboratory of Debrezeyt, Horticoop Ethiopia PLC. A composite of multiple samples of mungbean seeds was collected randomLy from local producers in Shewarobit, which is located 220 km north of Addis Ababa, Ethiopia. This crop was selected due to its large-scale production, consumption, and improper waste management, which leads to environmental pollution in the study area [23].

Apparatus, reagents, and chemicals

Digital balance, muffle furnace, pH meter, conductivity meter, ICP-OES, air circulating oven, and digestion tubes. The stock solutions of Cu, Cr, Pb (1000 mg/L, Certipur® Single-Element Standard for Inductively Coupled Plasma Spectroscopy (ICP)) were purchased, and a series of standard solutions for each element were prepared by diluting them. Analytical grade 70% nitric acid (HNO_3), hydrochloric acid (HCl), and 72% Perchloric acid ($HClO_4$) both were purchased.

Sample preparation

All glassware, containers, crucibles, mortars, and pestles were washed thoroughly with detergent, rinsed with distilled water, soaked in 10% HNO_3 solution for 24 hours [24], washed with distilled water, and dried in a drying oven at 105 °C for 5 hours.

The mungbean seeds were first washed with distilled water several times to remove all dirt particles and dried in the open air. The dried bean was then ground to a fine powder using porcelain mortar and pestle, sieved with a 2.0 mm sieve, and stored in a plastic bag (zip lock) at room temperature.

Sample digestion

Exactly 5.0 g of ground mungbean powder were weighed and transferred to a clean crucible, which is labeled according to the sample number in triplicate, and the dry ashing process was carried out in a muffle furnace by the stepwise increase of the temperature up to 550 °C and then left to ash at this temperature for 6 h. The sample was removed from the furnace and allowed to cool. The ash was wetted with water and 2.5 mL of concentrated HNO_3 was added. The crucible was covered with a watch glass and placed on the hot plate. The digestion was performed at a temperature of 90 to 95 °C for 1 h. The ash was dissolved in 5 mL of 9.25% HCl and digested again on a hot plate until the white fumes ceased to exist and the sample reached 2 mL. After cooling, 20 mL of distilled water was added and filtered using Whatman filter No.41 paper. The filtered sample was then diluted up the mark of 50 mL standard volumetric flask and stored at 4 °C temperature until analysis. Blanks were prepared to check for background

contamination by the reagents used [20,22,25].

Preparation of standard solution

A series of standard solutions (Table 2) were prepared from the stock solution (1000 mg/L) for each metal. Initial 10 mg/L concentration working solutions were prepared from the stock solutions by pipetting 1mL of 1000 mg/L into 1000-mL volumetric flasks and diluting to the mark with deionized water. Depending on the linear response range of the metal, the calibration standards ranging from 0.1 to 2.8 mg/L [14] were then prepared by appropriate, accurate, and precise dilution of the 10 mg/L working solutions. Then the determination of each element was done by using ICP-OES calibrated with a blank and series of each standard solution. Calibration curves were done for each element following the procedures mentioned elsewhere [18]. This technique can do multi elemental analysis with excellent sensitivity and high sample throughput, resulting in high precision and accuracy. The precision of the analytical method was evaluated in terms of repeatability of the experimental results of real samples and expressed as standard deviation (SD). The accuracy was verified by calibration (using standard solutions) [12].

Soil pH and electrical conductivity

The soil sample from which mung bean grows was collected using a polyethylene tube in the depth of around 40 cm following the scientific procedures of soil sampling. The pH and electrical conductivity of the soil were evaluated based on the method described [26]. The pH of the samples was determined with a handheld Hanna pH meter using the method described in [27]. Where 20 g of the dried soil was weighed into a 50 cm^3 beaker, 20 cm^3 of distilled water was then added. The mixture was stirred with a glass rod and allowed to stand for 30 minutes. A pre-calibrated pH meter was inserted into the slurry and the pH was recorded. The Electrical conductivity (EC) was determined using the modified method mentioned elsewhere [27]. 25 g of an air-dried soil sample was placed in a 250 cm^3 beaker, distilled water was added slowly drop by drop over the entire soil surface in a uniform pattern until the soil appeared wet. A stainless steel spatula was used to form a homogeneous soil paste. The beaker was then covered with a Petri dish, 50 cm^3 of distilled water was added and shaken for 1 hour. 40 cm^3 of the diluted extract was transferred into a 100 cm^3 beaker and the electrode of the conductivity meter was inserted to record the electrical conductivity of the soil recorded.

TABLE 1 Instrumental operating conditions for the determination of heavy metals using ICP-OES from mungbean seed

Instrumental parameter	Operating condition
Radiofrequency applied power (kW)	1.2
Plasma viewing mode	Axial
Plasma gas flow rate (L min-1)	12
Auxiliary gas flow rate (L min-1)	1
Nebulization gas flow rate (L min-1)	0.7
Pump speed (rpm)	12
Integration time (s)	5

Results and discussion

Soil pH and electric conductivity

The results demonstrated that the pH of the soil sample was 6.93 ± 0.153. and the electrical conductivity of the soil sample was 0.18133 dS/m ± 2.52). Soil samples are categorized as acidic (low pH), < 6.5, normal (medium pH): 6.5-7.8, and alkaline (high pH): >7.8 depending on the pH value. Depending on

electrical conductivity soil samples are also categorized as normal: < 0.8 dS/m, critical for salt-sensitive crops: 0.8-1.6 dS/m, critical to salt-tolerant crops: 1.6-2.5 dS/m and injurious to most crops: >2.5 dS/m. The experimental analyses showed that the pH of the soil sample was 6.93 ± 0.153 confirmed that the soil was normal (medium), which was weakly acidic and suited for mungbean production. Loam or semisandy soil having a pH value range from 6.2-7.3 supports the productivity of this crop. The experimentally analyzed pH of the sample soil was in this range by 6.93. The experimental analyses showed that the electrical conductivity of the soil sample was 0.18133 dS/m ± 2.52). The result confirmed that the soil sample was normal because its EC was fallen within the normal range of electrical conductivity (< 0.8 dS/m).

Instrument calibration

The instrument (ICP-OES) was calibrated using standard working series of solutions of each of the metals. The standard working solutions of each metal were prepared freshly by appropriate dilution of the intermediate standard solutions. Concentrations of the intermediate standards, working standard solutions, and values of correlation coefficients of the calibration graph for each of the metals are presented in Table 2.

TABLE 2 Concentration values of working standard solutions and correlation coefficients of calibration graph

Metals	Concentration of intermediate (mg/L)	Concentration of standards(mg/L)	Correlation coefficient of calibration graph
Lead (Pb)	10	0, 0.028, 0.056, 0.084, 0.28, 0.56, 0.84, 1.12, 1.4.	0.999757
Copper (Cu)	10	0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8.	0.999377
Chromium (Cr)	10	0, 0.028, 0.056, 0.084, 0.28, 0.56, 0.84, 1.12, 1.4.	0.999874

Analytical method detection limit

In general terms, the detection limit of an analytical method refers to the minimum concentration of a substance that can be reported with 99% confidence to be greater than zero [27]. Moreover, the limit of detection (LOD) is the smallest mass of analyte that can be distinguished from statistical fluctuations in a blank, which usually corresponds to the standard deviation of the blank emittance times a constant. The limit of detection is most commonly defined as the mass of the analyte that gives a signal equal to three times the standard deviation of the blank [27]. In this study, the detection limit for the method was calculated by multiplying the standard deviation of seven blank signals each determined in triplicate by three. The calculated LOD for the mung bean samples is given in Table 3. The method detection limit of each element is calculated using the formula.

Limits of Detection (LOD) = 3x SD of the blank [27]

MQL is the lowest concentration of analyte that can be measured in the 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. It is calculated as:

Quantification limit = 10 x Standard deviation of blank [27]

MQL is greater than the detection limit of the instrument, which confirmed that the method was available and the instrument better detected the concentration of the heavy metals in the sample [28]. The MQL was the least, which indicated that the accuracy is high. Therefore, the method (procedure) was more reliable.

TABLE 3 Detection and quantification limits of Pb, Cu, and Cr metals in mungbean

Metals	[a]MDL in mg/L± SD	[b]MQL in mg/L ± SD
Lead (Pb)	0.108 ± 0.036	0.36±0.036
Copper (Cu)	0.366 ± 0.122	1.22 ± 0.122
Chromium (Cr)	0.45 ± 0.15	1.5 ± 0.15

[a]Values are the mean of seven blank determinations, each measured three times
[b]Values are the mean of ten blank determinations, each measured three times

Evaluation of analytical method

Recovery tests using the proposed method were performed for the mung bean using non spiked and spiked samples, and each sample was determined in triplicate. As shown in Table 4, the results of percentage recovery for the studied metal in mung bean were all between 96% to 110.5%. The results of the recovery tests for the samples were within the acceptable range verifying the validity of the proposed method for mung bean analysis. These values were in agreement with the acceptable range of 80 to 120% expected for the elements, indicating good accuracy for the analysis procedure [32]. The calculation was done using the following equation.

$$Percentage\ recovery = \frac{Concentration\ in\ spiked sample - Concentration\ in\ unspiked sample}{Amount\ spiked} x\ 100 \quad [29]$$

TABLE 4 Percentage recovery of lead, copper, and chromium in mung bean

Heavy metals	[a]Concentration in sample (mg/L)	Amount Added in (mg/L)	[a]Concentration in spiked sample (mg/L)	Standard deviation	% [b]Recovery ± standard deviation
Copper (Cu)	6.53	0.5	7.08	0.039	110.5 ± 0.039
Lead (Pb)	2.51	0.5	2.99	0.236	96.0 ± 0.236
Chromium (Cr)	0.38	0.5	0.89	0.085	102.0 ±0.085

[a]Concentration values are the average of three analyzed samples ± standard deviation.
[b]Recovery values are mean ± standard deviation.

Levels of heavy metals in the mung bean

In this study, the concentration of lead, copper, and chromium was detected by ICP-OES in mung bean. The concentration of lead, copper and chromium in mung bean was 2.51 mg/L ± 0.236, 6.53 mg/L ± 0.039 and 0.38 mg/L ± 0.085, respectively (Figure 1). The permissible limit according to WHO standard (1996) is 2 mg/L ± 0.236, 6.53 mg/L ± 0.039, and 1.3 mg/L ± 0.085 for lead, copper and chromium, respectively. Based on the quantified concentration of target analyte in mung bean seed, the concentration of Cr<Pb<Cu. The study confirmed that the concentration of copper and chromium is below the standard which indicates the plant is safe for consumption. However, the concentration of lead is higher than the standards which hinder the productivity of the plant and risk the human health, this might be the cause for chronic exposure of Pb which could affect the physical growth of human beings and cause anemia, toxic musculoskeletal, renal, ocular, neurological, immunological, reproductive and developmental effects, kidney damage, headache, hearing problems, speaking problems, fatigue or irritable mood [20, 30]. This might be due to the accumulation of excess waste materials including animal carcasses wasted into the irrigation water hence, the local producers highly utilized polluted for irrigation. In addition to these, different fertilizers and used during crop productions may contribute to the accumulation of excess lead in mung bean seeds [31].

Although the concentration of copper in the sample was lower than the permissible limit, it

differs from previous research findings [32] due to the concentration of Cu differ according to the soil type and soil pollution. This might be due to the different waste materials which entered into the irrigation water and the fertilizers that farmers used might not be the cause for the increment of the concentration of copper in mung bean seed samples source [20]. Chromium is formed through natural processes and human activities. Chromium is not essential for plant growth, it was not detected in some plant sites since the uptake of chromium by plant shoot is generally low [33]. The data showed that the concentration of chromium in mungbean seeds was lower than the standard limit. In its normal concentration Cr is effective in the management of diabetes and it is a cofactor of insulin. The concentration level of chromium in sorghum, maize, wheat, and barley was found to be 0.95, 0.52, 0.43, and 0.29 mg kg−1 [34], which is lower than the permissible limit [20].

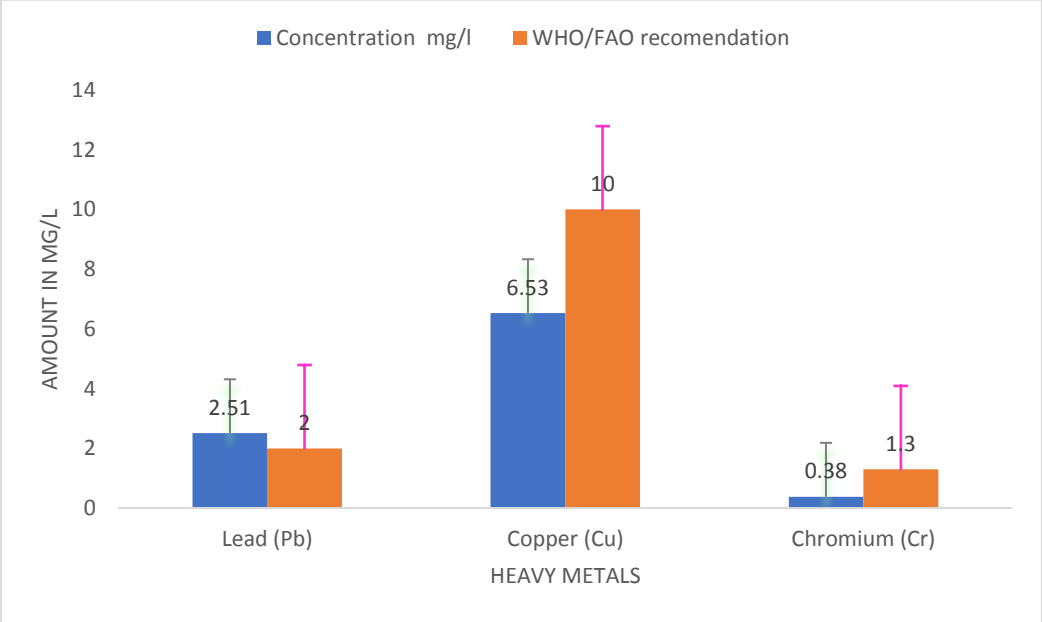

FIGURE 1 Concentrations of heavy metals (Pb, Cu, and Cr) as compared to with the recommended concentration by WHO/FAO in mung bean.

Conclusion

In this study, heavy metal accumulation (Pb, Cu, and Cr) in mungbean planted under irrigation was detected using an inductively coupled plasma optical emission spectrometer (ICP-OES). The study revealed that the concentrations of copper and chromium were under the permissible limit, but the concentration of lead is higher than the WHO recommendation limit and unsafe for consumption. Therefore, proper monitoring of effluents was necessary to avert excessive accumulation of toxic heavy metals in edible plants. Thus, the health risk and the extent of heavy metal contamination can be reduced.

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