Research article

Health risk assessment of heavy metals in selected Ethiopian spices

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ABSTRACT

A total of 144 spice samples (ajwain, black cumin, coriander and fenugreek) were collected from three districts. For the determination of heavy metals (Cd, Cr, Cu, Pb and Zn) concentrations and human health risks, the spice samples were digested in a mixture of HNO₃ and HClO₄. The highest concentrations of Cr (24.85 ± 1.673 mg/kg) and Cu (32.267 ± 7.355 mg/kg) were investigated in ajwain and Zn (54.13 ± 6.122 mg/kg) in fenugreek. However, lowest concentration of Cr and Cu were detected in fenugreek; and Zn in coriander. Cd and Pb were not detected in all spice samples. A positive correlation was observed among Cr, Cu and Zn in fenugreek; Zn with Cr and Cu in black cumin and Zn in coriander. However, negative correlation was observed Cr with Cu and Zn in ajwain and coriander; Cr with Cu in black cumin. THQ values of Cu, Zn and 75% of Cr in all spices were less than 1. About 66.7% of the HI values of the samples under study were less than 1, indicated that the consumers will not experience potential health risks from the intake of individual metals through spices consumption.

1. Introduction

Spices are natural food adjuncts that have been used for thousands of years to enhance sensory quality of foods. Various parts of spices like seeds, fruits and barks are primarily used as ingredients in cooking processes in order to impart aroma, color and taste of food and sometimes mask undesirable odors [1]. They are also valued for their coloring, as preservatives and fumigants, in pharmaceutical, textile and other industries [2, 3].

Ethiopia is an ancient country with suitable agro-ecologies for various agricultural products at various highlands and lowland areas, making it diversified for spices. Spices are mainly cultivated by small farmers, government owned plantations and in wild [4]. They are commonly used throughout the country as additives to cultural dishes, as well as for medicinal purposes [5]. Ginger, chillies, turmeric and black cumin are the most exported spices in the country. Many investigations have confirmed that spices such as ajwain, black cumin, coriander and fenugreek have shared around 36% in area coverage and 17% of the total spices grown in the country [6].

Heavy metals such as chromium, copper, iron, manganese and zinc are essential for humans, and they may play an important role in a number of biochemical processes [7]. These elements can be toxic to humans and other living when their intake exceeds certain threshold values. However, elements such as lead, cadmium, mercury and arsenic are non-essential and toxic even at low concentrations for humans [8, 9].

Recently, several attempts have been made to determine the metallic content of spices and herbs [10]. However, there were no report that revealed the levels of metals in spices grown from districts of Central Gondar Zone. Therefore, the main objective of this study was to determine the level of selected metals and to evaluate the potential health risk of these metals through the consumption of spices (ajwain, black cumin, coriander and fenugreek) which are widely grown in three districts of Central Gondar Zone.

2. Material and methods

2.1. Description of study areas

This research was carried out in three districts, namely: East Dembia, West Dembia and Takusa. The sampling areas are bordered by Lake Tana (the largest lake in Ethiopia) and the districts are also known by producing various fruits, vegetables and spices through irrigation in addition to the raining seasons. East and west Dembia districts are situated at 12°39’59.99" N latitude and 37°09’60.00" E longitude and has an elevation of 550–1600 m above sea level. However, Takusa is found with an elevation of 2133 m above sea level and lies at 12.0300’00” N latitude and at 36.940’00” E longitude.
Table 1. Description of spice samples.

| General name | Botanical name       | Family                  | Edible part |
|--------------|----------------------|-------------------------|-------------|
| Ajwain       | Trachyspermum ammi   | Apiaceae Umbellifere    | Fruit       |
| Black cumin  | Nigella sativa L     | Ranunculaceae           | Seed        |
| Coriander    | Coriandrum sativum L | Apiaceae                | Seed        |
| Fenugreek    | Trigonella foenum-graecum | Fabaceae               | Seed        |

For each district, three local markets were selected for spices sampling. These were Ayimba, Robit and Koladiba (East Dembia); Chenker, Choahit and Gawirma (West Dembia) and Delgi, Gundera and Mekonta (Takusa).

2.2. Sample collection

From each district, spice samples were collected from three different local markets. At each market four samples were bought and mixed together at each sampling point. A total of 144 samples were collected (3 districts x 3 local markets x 4 samples x 4 spices). The composite samples were packed in polyethylene plastic bags, labelled and transported to laboratory. In order to remove all soil and extraneous matter, the samples were washed thoroughly with tap water followed by deionized water. Then after, the cleaned spices were dried in an oven at 60 °C for 24 h. Finally, the dried samples were homogenized by grinding with a glass mortar and sieved through 2 mm nylon sieve to remove coarse debris. The botanical description of the spices used in this study are represented in Table 1.

2.3. Apparatus and chemicals

A drying oven was used to dry spice samples. Electronic grinder was used for grinding and homogenizing the spices. Analytical balance with a precision of ±0.0001 g was used to weigh the spices. Flame atomic absorption spectrophotometer (Buck Scientific Model 210VGP AAS, East Norwalk, USA) equipped with deuterium arc back ground correctors and hollow cathode lamps with air-acetylene flame was used for metals (Cd, Cr, Cu, Pb and Zn) analysis. All the reagents that used in the analysis were analytical grade. Deionized water was used to prepare and dilute standard solutions of metals and sample solutions throughout the study. HNO₃ (72%) and HClO₄ (70%) were used for digestion of spices. Stock standard solutions of 1000 mg/L Cd, Cr, Cu, Pb and Zn in 2% HNO₃ were used for preparation of standard solutions for calibration and in spiking experiments.

2.4. Heavy metal analysis

Prior to measuring the concentrations of Cd, Cr, Cu, Pb and Zn, a 0.5 g of powdered spices samples were mixed with various volumes of HNO₃ and HClO₄ and then digested at different digestion time and temperature depending the type of spices. The digestion procedures were optimized by varying the volume of acids added to the sample, the digestion temperature and time. A digestion procedure which has given clear and colorless solution at low reagents volume, low temperature and shortest digestion time was chosen as the optimal [11]. Ajwain was digested with a mixture of 5 mL of HNO₃ and 4 mL of HClO₄ at 240 °C for 1:45 h, black cumin with 5 mL of HNO₃ and 5 mL of HClO₄ at 240 °C for 2:10 h, coriander digested with a mixture of 5 mL of HNO₃ and 4 mL of HClO₄ at 240 °C for 2:15 h. Finally, fenugreek was digested with 5 mL of HNO₃ and 3 mL of HClO₄ at 240 °C for 1:0 h. The digestion was carried out in triplicate.

After digestion was completed, the solution was cooled and filtered out through Whatman No. 42 filter paper in to 50 mL volumetric flask and finally diluted with deionized water to the mark. Digestion of a reagent blank was also performed following similar digestion procedure as that of the sample. Finally, all digested solutions were kept in refrigerator until analyzed with flame atomic absorption spectrometry.

The flame atomic absorption spectrometry (FAAS) operating parameters for determination of metals were given in Table 2.

2.5. Method validation

Method validation is an analytical method that used to evaluate the reliability and repeatability of analytical results. Accuracy, precision, limit of detection, etc were used for validating analytical procedure [12]. Accuracy of an analytical method helps to ensure the loss or contamination occurred during preparation steps and matrix interferences [11]. Due to the absence of standardized certified reference materials, the accuracy of the method was validated by spiking experiments (recovery test). A recovery test was performed by spiking the spice samples with standard solutions of heavy metals. The spiked samples were digested with the optimized procedures applied for the unspiked spice samples. Precision value was estimated by calculating the standard deviation (SD) of the results of triplicate measurements for each sample [13]. Thus, in this study the degree of precision of the data are reported as mean ± SD of triplicate measurements.

Limit of detection (LOD) is defined as the minimum concentration of a substance that can be measured and it is the ratio of the value of three times the standard deviation of the blank sample to the slope of the calibration curve. Three replicate blank samples were digested following the same procedures utilized for digesting the spice samples [14, 15].

2.6. Health risk assessment of spices consumption

The potential health risks of heavy metal through spices consumption were assessed based on estimated daily intake (EDI) of metal, target hazard quotient (THQ) and Hazard Index (HI).

The EDI value depends on element concentrations in spices, the amount of daily consumption and body weight. EDI values of the analyzed metals were estimated based on Eq. (1).

Table 2. FAAS instrumental operating conditions of investigated elements.

| Element | Wave length (nm) | Lamp current (mA) | Slit width (nm) | Detection limit (mg/L) |
|---------|-----------------|-------------------|----------------|------------------------|
| Cd      | 228.9           | 2.0               | 0.7            | 0.01                   |
| Cr      | 357.8           | 2.0               | 0.7            | 0.05                   |
| Cu      | 324.7           | 0.7               | 0.7            | 0.005                  |
| Pb      | 283.3           | 2.0               | 0.7            | 0.04                   |
| Zn      | 213.9           | 2.0               | 0.7            | 0.005                  |
where, EDI is estimated daily intake and Cmetal (mg/kg) is an average weighted heavy metal content in spices, IR (ingestion rate) is the average long term consumption of spices (gram/day person), BW is the average body weight (Kg) \[16\]. The average IR of spices for adult is 10 g/day/person of dry weight which is similar to the literature. The average body weight for adult was 60.0 kg \[17\].

The HI has been developed to estimate the overall non-carcinogenic risk to human health through exposure of more than one pollutant. As shown in Eq. (2), THQ is used for assessment of non-carcinogenic risks associated with long term exposure of contaminants in vegetables and the calculations were made according Eq. (2).

\[
THQ = \frac{EDI}{RfD}
\]

where, RfD is reference dose values for each metals of interest (mg/kg day \(-1\)). The RfD values for Cr, Cu and Zn were 0.003, 0.04 and 0.3 mg kg \(-1\) per day, respectively \[18, 19\].

The HI has been developed to estimate the overall non-carcinogenic risk to human health through exposure of more than one pollutant. As shown in Eq. (3), HI is the total of the hazard quotients all heavy metals in spices.

\[
HI = THQ(Cr) + THQ(Cu) + THQ(Zn)
\]

If the values of THQ/HI \(\geq 1\) indicates that the population will pose potential adverse health effects, while if THQ/HI < 1, the population is unlikely to experience obvious adverse effect \[20, 21, 22\].

### 3. Results and discussion

#### 3.1. Method validation

As shown in Table 3, the average recoveries of metals in spice samples were ranged from 92.62% to 107.13%, indicating that the method was accurate and applicable for the determination of metals in spice samples \[23, 24\].

The precision of metal analysis has been estimated from the relative standard deviation (RSD) for three replicate analyses and it was found to be less than 10% for all metals. The LOD values were found to be 0.036 mg/L for Cd, 0.12 mg/L for Cr, 0.064 mg/L for Cu, 0.22 mg/L for Pb and 0.26 mg/L for Zn.

#### 3.2. Metals concentrations

Cadmium is highly toxic metal even at low concentration and accumulates in the kidneys and liver and it can cause many severe diseases \[25, 26, 27\]. The concentration of Cd in all spices was under the limit of detection of the applied method.

Chromium is a toxic heavy metal which plays important role for physiological and biological functions of the human body when it is below tolerable limits \[28\]. However, its deficiency causes hyperglycemia and elevated body fat; and high Cr concentration damages kidneys, liver, and blood cells through oxidation reactions \[26\].

### 2.7. Statistical analysis

Data were statistically analyzed using SPSS, version 20.0 and the values were reported as mean ± standard deviation). ANOVA analysis was implemented to test the differences among spices and between study regions. Differences are considered to be significant if \(p < 0.05\). Pearson correlation coefficients were also applied to investigate the correlations among metal concentrations. All the statistical tests were conducted at a 95% confidence level.

### Table 3. Recovery test for the optimized procedure of spices.

| Site           | Spice     | Metals | Recovery (%) | Cd        | Cr       | Cu        | Pb        | Zn        |
|----------------|-----------|--------|--------------|-----------|----------|-----------|-----------|-----------|
| East Dembia    | Ajwain    | ND     | 24.85 ± 1.672 | 17.63 ± 1.131 | ND       | 54.63 ± 8.41 | ND       |
|                | Black cumin | ND    | 11.2 ± 0.670 | 21.31 ± 1.213 | ND       | 52.38 ± 6.166 | ND       |
|                | Coriander | ND     | 18.93 ± 2.631 | 20.12 ± 1.642 | ND       | 19.14 ± 1.702 | ND       |
|                | Fenugreek | ND     | 16.14 ± 2.304 | 19.92 ± 2.702 | ND       | 54.13 ± 6.122 | ND       |
| West Dembia    | Ajwain    | ND     | 22.94 ± 3.063 | 20.26 ± 2.297 | ND       | 18.81 ± 1.906 | ND       |
|                | Black cumin | ND    | 11.64 ± 1.438 | 18.75 ± 3.767 | ND       | 31.38 ± 3.511 | ND       |
|                | Coriander | ND     | 12.35 ± 2.708 | 30.30 ± 2.268 | ND       | 14.24 ± 3.497 | ND       |
|                | Fenugreek | ND     | 11.07 ± 2.772 | 14.24 ± 3.497 | ND       | 41.07 ± 7.549 | ND       |
| Takusa         | Ajwain    | ND     | 14.62 ± 1.315 | 32.267 ± 7.355 | ND       | 46.34 ± 4.070 | ND       |
|                | Black cumin | ND    | 13.63 ± 0.791 | 13.98 ± 4.849 | ND       | 44.597 ± 4.000 | ND       |
|                | Coriander | ND     | 10.83 ± 1.019 | 21.53 ± 7.552 | ND       | 11.08 ± 1.918 | ND       |
|                | Fenugreek | ND     | 9.99 ± 1.614 | 11.083 ± 1.918 | ND       | 48.53 ± 0.790 | ND       |

Different letters in the same column are significantly different \((p < 0.05)\) in one way ANOVA.

### Table 4. Heavy metal concentrations in different spices (mean ± SD, mg/kg).

| Site           | Spice     | Metals | Cd       | Cr       | Cu       | Pb       | Zn       |
|----------------|-----------|--------|----------|----------|----------|----------|----------|
| East Dembia    | Ajwain    | ND     | 24.85 ± 1.672 | 17.63 ± 1.131 | ND       | 54.63 ± 8.41 | ND       |
|                | Black cumin | ND    | 11.2 ± 0.670 | 21.31 ± 1.213 | ND       | 52.38 ± 6.166 | ND       |
|                | Coriander | ND     | 18.93 ± 2.631 | 20.12 ± 1.642 | ND       | 19.14 ± 1.702 | ND       |
|                | Fenugreek | ND     | 16.14 ± 2.304 | 19.92 ± 2.702 | ND       | 54.13 ± 6.122 | ND       |
| West Dembia    | Ajwain    | ND     | 22.94 ± 3.063 | 20.26 ± 2.297 | ND       | 18.81 ± 1.906 | ND       |
|                | Black cumin | ND    | 11.64 ± 1.438 | 18.75 ± 3.767 | ND       | 31.38 ± 3.511 | ND       |
|                | Coriander | ND     | 12.35 ± 2.708 | 30.30 ± 2.268 | ND       | 14.24 ± 3.497 | ND       |
|                | Fenugreek | ND     | 11.07 ± 2.772 | 14.24 ± 3.497 | ND       | 41.07 ± 7.549 | ND       |
| Takusa         | Ajwain    | ND     | 14.62 ± 1.315 | 32.267 ± 7.355 | ND       | 46.34 ± 4.070 | ND       |
|                | Black cumin | ND    | 13.63 ± 0.791 | 13.98 ± 4.849 | ND       | 44.597 ± 4.000 | ND       |
|                | Coriander | ND     | 10.83 ± 1.019 | 21.53 ± 7.552 | ND       | 11.08 ± 1.918 | ND       |
|                | Fenugreek | ND     | 9.99 ± 1.614 | 11.083 ± 1.918 | ND       | 48.53 ± 0.790 | ND       |

Different letters in the same column are significantly different \((p < 0.05)\) in one way ANOVA.
Copper is also essential element which is important in variety of biochemical processes. However, at higher levels Cu is toxic and affects mainly the blood and kidneys [29, 30]. Lead is a non-essential heavy metal for any organism and can reach to human system through air, water and food. It has no beneficial role in human metabolism and produces a progressive toxicity even at lower levels and there is no known safe exposure level. It is accumulated in humans and animals mainly in the liver and kidneys [24, 31, 32].

Zinc is an essential element for normal growth and development in human beings. It can enhance the human immune system. High Zn concentration affects the respiratory, gastrointestinal and blood system causing nausea, vomiting, diarrhea, skin rashes, anemia and sterility. However, Zn deficiency may result in abnormal physiology disorders such as anemia, hypogonadism and dwarfism [9, 31].

The content of Cr in all spices samples differ significantly (p < 0.05) and found in range from 14.62 ± 1.315 to 24.85 ± 1.673 mg/kg, 11.2 ± 0.870 to 13.63 ± 0.791 mg/kg, 10.83 ± 1.019 to 18.93 ± 2.631 and 9.99 ± 1.614 to 16.14 ± 2.304 in ajwain, black cumin, coriander and fenugreek, respectively (Table 4). However, with the exception at ajwain, the concentration of Cr varied insignificantly (p > 0.05) in spices at the three study sites.

Ajwain collected from East Dembia contained significantly higher Cr content as compared with West Dembia and Takusa, which may be attributed to the difference in soil types and agricultural inputs. The concentration of Cr in fenugreek collected from West Dembia differ significantly (p > 0.05) with East Dembia and Takusa. Cr contents in black cumin, coriander and fenugreek are comparable with the results reported from Iraq [9], India [33] and Ethiopia [34]. But, the concentrations of Cr in black cumin were higher than reported from Ethiopia [4].

The mean concentration of Cu were found between 17.63 ± 1.131–32.267 ± 7.355 in ajwain, 13.98 ± 4.849–21.313 ± 1.213 mg/kg in black cumin, 20.12 ± 1.642–30.30 ± 2.268 in coriander and 11.083 ± 1.918–19.92 ± 2.702 in fenugreek.

Among all the analyzed spices, the highest and lowest contents of Cu were found in coriander (30.30 ± 2.268) and fenugreek (11.083 ± 1.918) at Takusa.

There were no significant differences (p > 0.05) observed in the availability of Cu in all the four spices. However, there were significant differences (p < 0.05) in Cu accumulation in ajwain and coriander between the three sampling sites.

Compared with findings of previously published articles, the concentration of Cu in ajwain, black cumin, coriander and fenugreek were found to be slightly higher than reported in Iraq [9], India [33], Pakistan [25] and Turkey [35]. However, the concentrations of Cu in fenugreek in this study were in agreement with the results reported in Ethiopia [34].

The concentrations of Zn investigated in this study were found in the range of (18.81 ± 1.906–46.34 ± 4.07), (31.38 ± 3.15–52.38 ± 6.166), (11.08 ± 1.918–19.143 ± 1.702) and (41.07 ± 7.34–54.13 ± 6.122) in ajwain, black cumin, coriander and fenugreek, respectively. Except in fenugreek the concentration of Zn in all spices collected from the different districts were varied significantly (p < 0.05). In this study, the highest concentrations of Zn (54.13 ± 6.122) were recorded in fenugreek at East Dembia and the least (11.08 ± 1.918) in coriander at Takusa. In addition, except in coriander the concentrations Zn were higher in comparison with the other studied metals.

When compared with the previous works, the concentrations of Zn in ajwain, were in agreement with the values reported by Batool and Khan from Pakistan [25]. Similarly, amounts of Zn in black cumin were in agreement with the results of other studies [9, 33]. The mean value of Zn recorded black cumin, however, higher than the values reported by Batool and Khan; and Al-Qahtani [25, 36].

Moreover, the concentrations of Zn in coriander were in agreement with the values reported from Ethiopia [4], while it was much higher than previously reported values from Iraq [9], India [33], Pakistan [25] and Turkey [35].

Zn was also present in appreciable amount in fenugreek (41.07 ± 7.34–54.13 ± 6.122 mg/kg) than the concentration reported [9, 25, 35, 37]. However, it was in agreement with other reports [34, 35, 38].

In general, the trend of average metal accumulation in spices were follow the order of: Zn > Cu > Cr in ajwain, black cumin and fenugreek. However, the order of metal concentration in coriander follow the order of: Cu > Zn > Cr.

The absence of Cd and Pb in all spices at the study areas prevailed that the spices cultivated are free from environmental pollution.

Pearson correlation matrix was applied in order to identify the associations between metal concentrations in spices. As shown in Table 5, a positive correlation was observed among all metals in fenugreek; Zn with Cr and Cu in black cumin; Zn with Cu in ajwain and coriander. However, negative correlation was observed Cr (with Cu and Zn) in ajwain and coriander; Cr with Cu in black cumin. According to pearson correlation relatively good positive correlation suggests the metals may arise from common sources as well as from similar in chemical properties [23]. The high negative correlation between metals on the other hand may indicate that the absorption of one metal by spice tends to decrease absorption of other metals [39].

As shown in Table 5, Zn showed a significant positive correlations with Cr in black cumin and fenugreek. However, Zn showed significant

| Table 5. Pearson’s correlation matrix for heavy metal in spices. |
|-----------------|-------|-------|
|                 | Cr    | Cu    | Zn    |
| Ajwain          | Cr    | 1.00  | Cu    | -0.812 |
|                 | Cu    | -0.812| 1.00  | 0.515  |
|                 | Zn    | -0.650*| 0.515| 1.00   |
| Black cumin     | Cr    | 1.00  | Cu    | -0.288* |
|                 | Cu    | -0.288*| 1.00  | 0.258* |
|                 | Zn    | 0.211* | 0.258*| 1.00   |
| Coriander       | Cr    | 1.00  | Cu    | -0.113  |
|                 | Cu    | -0.113 | 1.00  | 0.734* |
|                 | Zn    | -0.435*| 0.734*| 1.00   |
| Fenugreek       | Cr    | 1.00  | Cu    | 0.644  |
|                 | Cu    | 0.644  | 1.00  | 0.667* |
|                 | Zn    | 0.302* | 0.667*| 1.00   |

* Significantly different (p < 0.05) in one way ANOVA.
negative correlations with Cr in ajwain and coriander; and with Cu in black cumin, coriander and fenugreek. Cu showed significant negative correlations with Cr in black cumin.

There were insignificant correlations between Cu with Cr in ajwain, coriander and fenugreek. There were insignificant correlations between Cu with Zn in ajwain.

### 3.3. Health risk assessment

As shown in Table 6, the EDI values of Cr, Cu and Zn were calculated to be 0.0002–0.0041, 0.0018–0.0270 and 0.0002–0.0090, respectively.

The THQ values were ranged from 0.008–1.3805 for Cr, 0.0499–0.1453 for Cu and 0.0062–0.0301 for Zn. Except the THQ values of Cr in ajwain collected from East Dembia and West Dembia; and coriander collected from East Dembia, other THQ values in all spices were less than one, indicating that consumption of spices do not impose a potential health risk from Cr.

It can be seen that 66.7% of the HI values due to the consumption of spices were less than one. In general, serious chronic health impact has been suggested when the value of HI is > 10. Therefore, THQ and HI values suggested that the exposed population is assumed to be safe.

### 4. Conclusion

In conclusion, this study was aimed to investigate the concentration of different metals in Ethiopian spices. The optimized wet digestion method was applied for metals analysis in spices and it was validated through the recovery experiment and good percentage recoveries were obtained (92.62–107.13%). The results of this study showed that the highest amount of Cr was obtained in ajwain and the least at fenugreek, while Cu and Zn were detected in coriander and the least at black cumin and ajwain, respectively. Cd and Pb were found below detection limit in all investigated spices. Zn was the most abundant metal among the tested metals. The difference in the content of metals in each spice may be attributed the difference in soil types and agricultural inputs.

The results of the present study indicate that Cr is the only metal in all spices slightly contributes non-carcinogenic risk from the point of view of THQ. In general, the findings of the study show low health risk of the metals Cr, Cu and Zn through consumption of these spices.

### Declarations

**Author contribution statement**

Molla Tefera: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Almaz Teklewold: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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**Data availability statement**

The authors do not have permission to share data.

**Declaration of interests statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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