Levels and health risk assessment of trace metals in honey from different districts of Bench Sheko Zone, Southwest Ethiopia

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ABSTRACT

This study aimed to determine the levels and potential health risks posed by trace metals (Zn, Cu, Mn, Cd, Cr, and Pb) obtained in honey samples. The honey samples were from Sheko, Guraferda, Mizan Aman Town, Debub Bench, and Semien Bench in Bench Sheko Zone, Southern Ethiopia, and levels of trace metals were determined using atomic absorption spectroscopy (AAS). The levels of trace metals in honey samples across the provinces ranged as follows; Zn (1.78–4.02 mg/kg), Cu(1.85–2.35 mg/kg), Mn (0.75–1.25 mg/kg), Cd (0.011–0.038 mg/kg), Cr (0.25–0.55 mg/kg), respectively. The level of Pb was not detected in all honey samples. The levels of trace metals obtained were lower than the maximum permissible limit set by WHO/FAO. Limit of detection (LOD) and quantification (LOQ) were ranged from 0.00045 to 0.005 and 0.0015 to 0.016, for the tested metals, respectively. The estimated recoveries of the method were ranged from 92.66% to 103.00% showed a good agreement of accuracy. The EDI values (mg/kg) of the investigated trace metals in all honey samples were less than the maximum tolerable daily intake (MTDI). The values of the target hazard quotient (THQ) and the hazard index (HI) were less than unity. The estimated cancer risk (TCR) values of Cd in all honey samples showed the lowest carcinogenic risks with values ranged from 3.34E-07 to 1.27E-06. However, the TCR value of Cr in all honey samples ranged from 1.03E-05 to 2.43E-05 and was in the moderate range. The TCR value of Cd and Cr ranged from 1.15E-05 to 2.37E-05 and 1.23E-05 to 2.55E-05 for male and female adults, respectively, and were found in the moderate range. Therefore, there was no serious non-carcinogenic and carcinogenic risks to human health from exposure to trace metals through the consumption of this honey.

1. Introduction

The honeybee is an effective pollinator, essential for crop pollination and biodiversity conservation worldwide, both economically and agriculturally. Honeybee pollination benefits one-third of all food crops worldwide [1]. Ethiopia is domestic to numerous flora and fauna among African countries due to various ecological and climatic situations [2]. Within Ethiopia, the Kafka, Sheka, and Bench Sheko Zone (Southern Nations, Nationalities, and Peoples Region (SNNPR)) have a high potential for organic honey production with dense forest cover, which has been given priority as a region. Likewise, the demand for natural honey harvested in those regions is nowadays developing inside the home and export markets and can supply 500,000 tons of honey. However, modern-day manufacturing is restricted to 43,000 tons of honey [3].

Honey is a herbal supersaturated sugar solution and a sweet viscous semi-liquid product produced by bees from the nectar of vegetation. Honey is considered one of the oldest assets of nutrients for people and is famous around the globe [4]. In addition, it contains various minor ingredients, including enzymes (glucose oxidase, catalase, and phosphatases), glucose and sucrose (65–75% of total soluble solids), proteins, amino acids, organic acids, vitamins, lipids, volatile chemicals, flavonoids, phenolic acids, and minerals. Honey's biochemical properties, as well as its quality, are influenced by its ripeness, climatic conditions, production methods, processing conditions, and storage conditions [5, 6, 7].

Honey can scavenge free radicals from the human body because of its antioxidant properties. It is one of nature's gifts that has benefited people for centuries for its medicinal and therapeutic abilities, consumed alone or in combination with other substances for different purposes and treatments. Honey's antibacterial properties allow it to heal wounds, and it can also cure diseases such as diabetes, pressure, leg ulcers, burns, and cuts [6].

The USEPA has listed trace metals as pollutants with priority since the 1970s. Trace metals have been identified as a public health concern...
worldwide. Because of their extensive presence in diverse environments contributes to mutagenesis, carcinogenesis, and teratogenesis. During a risk assessment, consider the links between pollution sources, pathways, and receptors to evaluate the potential hazards associated with contaminated sites or products. It is possible to dissociate these linkages by removing sources, managing pathways, or modifying receptor exposure. To assess the health risk of trace metals is very helpful to have information about their concentrations in foodstuffs and their dietary intake [7].

There are various sources of trace elements in the environment (soil, air, and water), ranging from natural processes like forest fires, sea spray, and soil dust to anthropogenic activities such as agrochemical use, coal combustion, and mineral processing, traffic emissions, and sewage sludge discharges. During foraging, worker honeybees come into contact with soil and dust contaminant particles that adhere to their bodies and hairs. Some studies have indicated that honey may contain excessive trace elements, such as Cd, Cr, Cu, Pb, and Zn, which were introduced into the beehive mainly through the use of corrosive tools and containers during harvesting and processing [1].

Honey is a known nutritional food, and determining its trace metal content is imperative for quality control [8]. Trace metals are the most critical pollutant issues for the surroundings and are not biodegradable. Therefore, they can accumulate in vital human organs through the soil, skin contact, respiration, and the food chain and cause progressive toxic effects [9]. For example, trace metals can affect the quality of life when they accumulate in the human body and pose a risk to consumer health [6]. Exposure to trace and toxic metals negatively affect the quantity and quality of crops and cause damage to human health throughout the food chain [10].

The risk assessment method is used in risk analysis to determine if exposure to a hazardous chemical will cause harm based on the scientific procedure. As a result, human health risk assessment requires identifying, collecting, and integrating information on the health hazards of chemicals, human exposure to toxic compounds in polluted environment media, and the correlations between exposure duration, dose, and adverse health effects [10]. Identifying hazards, analyzing dose-responses, assessing exposure, and evaluating risks comprise the assessment of potential human health risks [11, 12]. For safe consumption of honey, it is crucial to consider the trace metals in the product and their associated health risks.

Bench Sheko Zone is one of potentially honey production province in the southwest Ethiopia because of favourable climatic conditions. In the province the consumption of honey by the community is high. Honey plays both a nurturing and therapeutic role, so toxic chemicals must be monitored continuously. There is no reported information in the literature concerning honey consumption in the province that is safe. As trace and toxic metal pollution affect honey quality, it can harm human health through the food chain. Therefore for the safe consumption of honey, the presence of trace metals in this product and the associated health risks need to be studied. Therefore, the main objective of this study was to determine the concentrations of trace metals (Cr, Mn, Cu, Zn, Pb, and Cd) and associated health risks of trace metals in honey from five different districts of Bench Sheko Zone.

2. Materials and methods

2.1. Description of study areas

The Bench Sheko/Maji zone has a total area of 24,554.16 square kilometers. It is astronomically located between 5.33° and 7.21° north latitude and 34.88°–36.14° east longitude with an elevation of 374–2,639 m above sea level. It is located in the SNPNPR in the Southwestern part of Ethiopia. Bench Sheko Zone borders the Elemi Triangle on the south, Sudan on the west, Gambela Region on the northwest, Kefficho Shekicho on the north, and Debub Omo on the east. It includes eleven districts (Sheko, Guraferda, Debub Bench, Shay Bench, Meinit Goldeya, Meinit Shasha, Maji, Bero, Surma, Semien Bench, and the city of Mizan Aman Town). The selected study areas were presented in Figure 1. In terms of agroecology of the region, 28.04%2 of the total land area is Kolla, 15.44% Weinadega, and 56.74% dega. The predominant food plants in this vicinity are corn, godere (taro root), and enset, while sorghum, teff, wheat, and barley are also very appreciated. Cash crops include fruits (bananas, pineapples, oranges, mango, and avocado) and spices (coriander and ginger) widely cultivated; Honey is also an important local source of income [13].

2.2. Chemicals and apparatus

All reagents used within the evaluation were of analytical grade. All the solutions used throughout the analyses were prepared with deionized water made at our laboratory. HNO3 (69.72%) and HClO4 (70%) were used for the digestion of honey. 1000 mg/L solutions of Cr, Mn, Cu, Zn, Pb, and Cd in 2% HNO3 have been employed for the preparation of the solutions used throughout the analyses for calibration and further experiments. The weight of honey samples was measured using an electrical balance with a precision of ±0.0001 g. For metals analysis, Atomic Absorption Spectroscopy (BUCK SCIENTIFIC MODEL 210VGP) geared up with deuterium arc background correctors and an air-acetylene flame hollow cathode was used.

2.3. Sample collection

For this study, the research sites from the Bench sheko zone were selected purposively by considering honey production. Among the five sites, Mizan Aman town is the center for all Weredas in the zone and the different types of honey were delivered to the Town from all areas of the Zone. At this site, the honey samples were collected from Aman and Mizan and pooled together to make a composite sample. The five honey samples were collected with 15 repetitions of 50g for each type of honey (total mass of the composite was about 0.750kg) of wild honey were brought by honey collectors from five different districts in the Bench Sheko Zone from October 2021 to April 2021 using polyethylene bottles. The samples were stored in a refrigerator until analysis. All-natural honey samples examined were raw kinds of honey from randomly mixed plant species.

2.4. Trace metal concentrations analysis

0.5g of honey samples were weighed with an electrical analytical balance and transferred to beakers to measure Cd, Pb, Cr, Cu, Mn, and Zn concentrations. Different volumes of HNO3, H2O2, and HClO4 were mixed with the samples and digested at varying times and temperatures. To optimize digestion procedures, digestion reagents, temperature, and time were varied. As a result, digestion was selected as the best method, resulting in clear and colorless solutions with a small reagent volume, at a low temperature, and with the shortest digestion time needed [14].

Honey samples were digested with a mixture of 9 ml HNO3 and 3 ml H2O2 at 130 °C for 2:30hr (optimal conditions). After digestion, the solutions were cooled, filtered through Whatman No.1 filter paper, and diluted with deionized water in a 50 ml volumetric flask.

The blank digestions were performed the same way as the sample digestions [17]. The reagents used were checked for background contamination by preparing blank samples. Honey samples were digested three times together with blank samples to minimize errors. The atomic absorption spectrophotometer was calibrated with a series of standard solutions and used to analyze trace metals in honey samples.

2.5. Method validation

Appropriate quality assurance procedures and precautions were considered and performed to confirm the reliability of the results. Calibration for quantification of concentrations of trace metals was...
performed by preparing standards at concentrations of 0.01, 0.2, 0.4, 0.6, 1.2, and 2.4 mg/L from 10 mg/L intermediate standard solutions (Table 1). The analytical method for the quantitative chemical analysis of trace metals in honey samples was validated by: selective evaluation, linear and working ranges, LOD and LOQ values, repeatability, and reproducibility (precision). Method specificity/selectivity (absence of interference), precision, and recovery of spiked standards calculated within the described calibration range [15, 16].

2.6. Health risk assessment

Risk assessment is the method of assessing the likelihood that a specified number of likely adverse health effects will occur over a specified period. During a health risk assessment, contaminants are evaluated for their potential carcinogenicity or non-carcinogenicity [17].

Human health risk assessments of chemical compounds are crucial to evaluate past, present-day, and even destiny exposures to hazardous chemicals and other chemical compounds. They can be quantitative or qualitative. Often lack of comprehensive information analysis of risk assessment is limited. Regardless, chemical risk assessments are investigated based on scientific knowledge about the behavior, exposure, dose, and contamination of pollutants toxicity levels. In general, risk depends on the following factors: (1) the amount of a chemical existing in the surrounding intermediates (e.g., soil, water, air), food, or a product; (2) the extent of a person’s contact (exposure) to the environmental pollutant; and (3) the toxicity of the chemical [10].

2.6.1. Calculation of daily intake of metals

The estimated daily intake of metals (EDI) was calculated using Eq. (1) [18]:

$$\text{EDI} = \frac{\text{EF} \times \text{ED} \times \text{IR} \times \text{Cm}}{\text{BW} \times \text{TA}}$$

Where EF = exposure frequency (365 days/year); ED exposure period (for male adults 60 years, female male-female adults 64 years) [19] considered for this study, which is equivalent to the average life span; IR is the honey consumption rate per day (g/day/person), the rate of consumption of honey per day could influence the tolerance of metal contaminants. Honey was thought to be a commonly consumed honeybee product in Ethiopia, with an average daily intake of 5.5 g previously reported by Mulugeta et al., [20]; Cm = metal concentration (mg/kg dry weight); BW is the adults’ average body weight which is considered (67
and 62.3 kg for male and female adults, respectively) as reported by [21]; and TA average exposure period (EDx365 days/year).

### 2.6.2. Target hazard quotient of metals

The target hazard quotient (THQ) factor measures the likelihood of non-carcinogenic risk from a potentially toxic trace element based on the oral reference dose (RfD o) [17]. The target hazard quotient (THQ) was calculated based on the methods [10, 21] described by Eq. (2):

\[
\text{THQ} = \frac{\text{EDI}}{\text{RfD}_0}
\]  

(2)

Where RfD<sub>0</sub> represents the oral reference dose (mg/kg/day) for adults and EDI is the estimated daily intake of metal in mg/day/kg body weight.

The reference dose for Cd, Pb, Cu, Mn, Zn, and Cr was presented in Table 2 [4]. If the values of THQ are greater than 1, the consumption of honey is safe for human health. If its value is less than 1, the consumption of honey is safe for human health. If its value is less than 1, there is an insignificant risk of non-carcinogenic effects [9].

### 2.6.3. Target cancer risks (TCR)

The TCR is used to evaluate the potential risk related to publicity to carcinogenic dealers for the duration of the lifetime exposure period [22]. The TCR values obtained using the Oral Cancer Slope factor (CPSo) in (mg/kg/day)<sup>-1</sup> presented in Table 2, which is the risk produced by a lifetime average dose in mg kg<sup>-1</sup> BDay<sup>-1</sup> and is contaminant specific [22, 23, 24, 25] and it was calculated by Eq. (4):

\[
\text{TCR} = \text{EDI} \times \text{CPSo}
\]  

(4)

The overall cancer risk because of exposure to more than one contaminant taken from a specific honey sample can be assumed to be the sum of each metal incremental lifetime most cancers risk (ΣTCR, n = 1, 2, 3, …, n).

### 2.7. Statistical analysis

First, the Shapiro-Wilk test was applied to determine the normality of distribution in the obtained data. Next, Significant statistical differences (P < 0.05) were determined using Duncan's test by one-way analysis of variance (ANOVA) followed by post hoc and Tukey HSD tests at a 95% confidence level using SPSS.20 software. The mean levels of elements were compared with maximum permissible limits using a one-sample t-test. In addition, we used a 2-tailed Pearson correlation test to investigate the correlation of heavy metal concentrations in honey samples.

### 3. Results and discussion

#### 3.1. Method validation

The value of precision was estimated by calculating the standard deviation (SD) of the results of triplicate measurements for each sample [26]. The mean values were determined from triplicate analysis of each sample. The accuracy and precision of the results were checked by different statistical methods after the determination of the levels of

### Table 1. Instrumental working condition for determination of metals in a honey sample by using FAAS.

| Metals | \(\lambda\) (nm) | Working standards (ppm) | Regression Eq. | R² |
|--------|----------------|------------------------|----------------|----|
| Zn     | 213.9          | 0.01, 0.2, 0.4, 0.6, 1.2, 2.4 | \(y = 0.165x + 0.024\) | 0.996 |
| Cu     | 324.7          | 0.01, 0.2, 0.4, 0.6, 1.2, 2.4 | \(y = 0.057x + 0.002\) | 0.997 |
| Mn     | 279.5          | 0.01, 0.2, 0.4, 0.6, 1.2, 2.4 | \(y = 0.064x + 0.001\) | 0.999 |
| Cd     | 228.9          | 0.01, 0.2, 0.4, 0.6, 1.2, 2.4 | \(y = 0.171x + 0.000\) | 0.996 |
| Pb     | 283.9          | 0.01, 0.2, 0.4, 0.6, 1.2, 2.4 | \(y = 0.055x + 0.002\) | 0.993 |

### Table 2. Exposure parameters are used for the health risk estimations by honey consumption.

| Parameters                      | Unit     | Male adults | Female adults | References |
|---------------------------------|----------|-------------|---------------|------------|
| Body Weight                     | Kg       | 67          | 62.3          | [21]       |
| Exposure Frequency (EF)         | Days/years | 365         | 365           |            |
| Exposure duration (ED)          | Years    | 60          | 64            | [19]       |
| Ingestion rate (IR)             | Kg/person/day | 5.5 x 10⁻³ | 5.5 x 10⁻³   | [20]       |
| Average time (TA) For non-carcinogenic | Days/years | 365 x ED    | 365 x ED     | [23, 26]   |

### Table 3. Recovery test for the optimized procedure of honey sample.

| Metals | Amount before spiked (mg/kg) | Amount added (mg/kg) | Amount obtained (mg/kg) | Recovery (%) | LOD | LOQ |
|--------|-----------------------------|----------------------|------------------------|--------------|-----|-----|
| Zn     | 3.25 ± 0.11                 | 3                    | 6.12 ± 0.02            | 95.66 ± 1.23 | 0.00210 | 0.007 |
| Cu     | 1.85 ± 0.03                 | 2                    | 3.91 ± 0.01            | 103.00 ± 0.52 | 0.00500 | 0.016 |
| Mn     | 0.75 ± 0.12                 | 2                    | 2.68 ± 0.04            | 96.50 ± 1.26 | 0.00460 | 0.015 |
| Cd     | 0.017 ± 0.004               | 1                    | 1.016 ± 0.06           | 99.90 ± 2.51 | 0.00031 | 0.001 |
| Cr     | 0.35 ± 0.02                 | 1.5                  | 1.74 ± 0.02            | 92.66% ± 0.25 | 0.00045 | 0.0015 |
metals in the honey samples. As indicated in Table 3, the estimated LOD and LOQ values had been obtained to be 0.0021 and 0.007 mg/L for Zn, 0.005 and 0.016 mg/L for Cu, 0.0046 and 0.015 mg/L for Mn, 0.0031 and 0.001 mg/L for Cd and 0.00045 and 0.0015 mg/L for Pb, respectively for triplicate analysis.

The recovery analysis was conducted by spiking honey samples for Zn, Cu, Mn, Cd, and Cr to ensure the reliability of the results. As shown in Table 3, the average recoveries of trace metals in honey samples had been confirmed with good accuracy ranging from 92.66% to 103.00%, indicating that the approach became accurate and applicable for analysis of trace metals in honey samples [7, 26].

### 3.2. Levels of trace metals in honey samples

The levels of trace metals with standard deviations for the examined honey samples were presented in Table 4. With an average value of 2.98 mg/kg, Zn is the most abundant metal in honey samples, followed by Cu, which has an average result of 2.036 mg/kg. The results obtained showed that the levels of Zn in honey samples were in the range of 1.78–4.02 mg/kg, Cu ranged from 1.85–2.35 mg/kg, Mn in the range of 0.75–1.25 mg/kg, Cd in range of 0.011–0.038 mg/kg, and Cr in the range of 0.25–0.55 mg/kg, respectively. However, the level of Pb in all examined honey samples was not detected because its value in samples was below the detection limit. The levels of trace metals in honey samples were found in the increasing order of Zn > Cu > Mn > Cd > Cr. The highest concentration was measured for Zn in all collected samples except the sample collected from Mizan Aman Town (Table 4). The highest concentration of Zn (4.02 ± 0.15 mg/kg) was obtained from Semien Bench and the lowest concentration of Cd (0.011 ± 0.005 mg/kg) was from Debub Bench. The trace metals of Zn, Cu, and Cd from Semien Bench, Mn from Guraferda, and Cd from Mizan Aman Town showed the highest concentration in honey samples presented in Table 4. Cd is found in soil, which is passed on to plants and nectar, and it is regarded as a bioindicator for honey contamination [27].

Based on the results of the One-way (ANOVA) analysis, significant differences (P < 0.05) were observed among the concentrations of trace metals (mg/kg) of (Zn, Cu, Mn, and Cd). However, the results of Cr from Sheko and Debub Bench and guraferda and Mizan Aman Town were not significantly different (P > 0.05).

A comparison of the levels of trace metals in examined honey samples was within the maximum permissible limit (MPL). Therefore, the maximum allowable limits are (0.2 mg/kg for Cd, 1.5 mg/kg for Cr, 10 mg/kg for Zn, 5.5 mg/kg for Mn, and 3 mg/kg for Cu) [24, 28] established by FAO/WHO [29] revealed that the mean levels of Cd, Cr, Mn, Cu, and Zn were lower than MPL. The results of one sample t-test showed that the mean concentration of trace metals obtained from honey samples were significantly lower (p < 0.05) than the maximum permissible limits established by FAO/WHO.

The data presented in Table 5 show comparison of the levels of metal reported in the literature with the levels found in this study. As compared to the concentrations of Cu (18.58 mg/kg), Cd (48.54 mg/kg), and Cd (5.83 mg/kg) reported from Iran [6] were significantly higher than in the present study. The levels of Cu (0.02–0.05 mg/kg), Cr (0.002–0.02 mg/kg), and Zn (0.17–0.64 mg/kg) reported from Nigeria [7] were lower than the results reported in the current study. According to the current study, the levels of Cd (0.011–0.098 mg/kg) found in Nigeria agreed with the present results. The content of Mn obtained in the present study was found in the range of results obtained (0.44–3.17 mg/kg) from honey

| Location          | Metals | References |
|-------------------|--------|------------|
|                   | Zn     | Cu         | Mn     | Cr     | Pb     | Cd     |
| Iran              | 0.17–0.64 | 0.002–0.05 | 0.002–0.02 | 0.005–0.081 | 0.011–0.098 |
| Nigeria           | 0.002–0.015 | 0.39–0.52 | 0.16 | 0.01 |
| Pakistan          | 1.11–4.1 | 0.08–0.33 | 0.12–0.95 | 0.01–0.10 | 0.01–0.14 | 0.01–0.38 |
| Saudi Arabia      | 0.004–0.11 | 0.005 | 0.015 | 0.1025 | 0.073 | 0.0115 |
| Romania           | 0.033 | 0.08–0.33 | 0.12–0.95 | 0.01–0.10 | 0.01–0.14 | 0.01–0.38 |
| Bangladesh        | 4.2–8.1 | 0.95–19.6 | 0.09–0.5 | 0.1025 | 0.073 | 0.0115 |
| Ghana             | 1.92–4.22 | 0.468 | 0.885 | 1.2–4.33 | ND | 0.69 |
| Ethiopia          | 9.96–16.03 | 0.02–1.15 | 0.15 | 2.53 | 0.017 |
| Ethiopia          | 0.725 | 0.794 | 0.021 | 0.25–0.55 | ND | 0.011–0.038 |
| Ethiopia          | 1.78–4.02 | 1.85–2.35 | 0.75–1.25 | 0.25–0.55 | ND | 0.011–0.038 |

### Table 4. Concentrations of trace metals in honey samples (mg/kg) (mean ± SD, n = 3).

| Sample Sites  | Metal Concentration |
|---------------|---------------------|
|                | Zn                  | Cu                  | Mn             | Cd                  | Pb               | Cr            |
| Sheko         | 3.25 ± 0.11a       | 1.85 ± 0.03a       | 0.75 ± 0.12a   | 0.017 ± 0.004a     | ND               | 0.3 ± 0.02a   |
| Guraferda     | 2.98 ± 2.05b       | 1.97 ± 0.06b       | 1.25 ± 0.51b   | 0.025 ± 0.001b     | ND               | 0.27 ± 0.05b  |
| Debub Bench   | 2.44 ± 0.18c       | 1.93 ± 0.12c       | 1.05 ± 0.25c   | 0.011 ± 0.005c     | ND               | 0.34 ± 0.08c  |
| Mizan Aman T. | 1.78 ± 0.08d       | 2.08 ± 0.32d       | 0.98 ± 0.06d   | 0.038 ± 0.006d     | ND               | 0.25 ± 0.03c  |
| Semien Bench  | 4.02 ± 0.15e       | 2.35 ± 0.16e       | 0.89 ± 0.32e   | 0.035 ± 0.007e     | ND               | 0.55 ± 0.09e  |
| Min           | 1.78               | 1.85               | 0.75           | 0.011              | ND               | 0.25          |
| Max           | 4.02               | 2.35               | 1.25           | 0.038              | ND               | 0.55          |
| Mean (SD)     | 2.89 (0.84)        | 2.04 (0.19)        | 0.98 (0.18)    | 0.025 (0.012)      | ND               | 0.35 (0.12)   |
| Median        | 2.98               | 1.97               | 0.98           | 0.025              | ND               | 0.34          |
| CV(%)         | 29.06              | 9.31               | 18.37          | 44                 | ND               | 34.28         |

Different superscript letters within same columns indicate significant differences by Tukey’s post-hoc tests (p < 0.05) and ND = Not Detected.
samples from Nigeria [7]. According to Ernest et al., report the mean concentration of Zn (40.06 mg/kg), Cu (58.11 mg/kg), Cr (6.67 mg/kg), and Cd (0.088 mg/kg) from Nigeria [30] was much higher than the present study.

The mean concentrations of trace metals in Ethiopia reported by [31] of Zn (1.10–4.22 mg/kg) and Mn (0.16–0.88 mg/kg) showed comparable results. In contrast, Cd concentrations (0.19–0.54 mg/kg), Cr concentrations (1.20–4.33 mg/kg), and Cu concentrations (0.09–0.46 mg/kg) were slightly lower than this study. The levels of Zn and Mn in the current study were within good agreement with the results reported from pakistan [32]. However, the levels of Cu and Cr in the same report were lower than in the current study, as illustrated in Table 5. The levels of Zn (1.70 ± 0.74 mg/kg) and Cr (0.042 ± 0.038 mg/kg) reported from Saudi Arabia [33] were lower than in the current study. As illustrated in Table 5, the levels of all trace metals (Zn, Cu, Mn, Cu, Cd, Cr, and Pb) reported from Ghana [28] were higher than in the present study.

The mean levels of Zn, Cu, and Cd obtained from the current study were lower than other reported values for honey from Romania [34], illustrated in Table 5. The levels of Zn in this study were in good agreement with those reported in Ethiopia [31]. However, it was lower than reported before [35] and higher than reported by others [36]. Compared to Bangladesh [37] and Ethiopia [31, 35, 36], the present study found higher Cu concentrations. The level of Mn obtained from Bangladesh [37] (2.69–3.49 mg/kg) and Ethiopia [35] (7.29 mg/kg) was higher than the result obtained in the current study. The levels of Cr and Cd reported in this study were lower than those obtained from Ghana [28] and Ethiopia [31]. The content of Cr reported by another study in Ethiopia [35] was lower than the results reported in the present study, and the values of Cd in the same study were in good agreement.

The levels of Pb reported by other studies [6] Iran [7, 30], Nigeria [32], Pakistan [33], Saudi Arabia [34], Romania [37], Bangladesh [38], Ghana, and [35] Ethiopia, but its concentration was not reported in Ethiopia [31, 36] and in the present study. There were some degrees of disagreement between the findings in our surveys and those in the surveys. These may be the result of differences in the studied regions. Despite this, poor harvesting and storage conditions and hygienic violations during fumigation, extraction, and storage may increase trace metal levels in honey [34]. The difference in the metal concentrations in the honey samples produced from the study areas could be attributed to differences in the botanical and geographical origins of the honey. The bee-keeping practices, environmental pollution, and honey processing also contributed to the diversified mineral contents found to be present in honey samples [30].

For metals determined in the samples, Pearson’s correlation matrices were used to correlate the effects of one metal on the other metal based on correlation coefficients (r) [36, 38] and presented in Table 6. Pearson correlation matrices indicated that most trace metals have negative correlations. Cd with Zn and Cr with Cd show low positive correlations. These weak correlations revealed that each metal in the samples affects the other metals to a lower extent and indicates that most metals have different sources of accumulation in honey. However, a strongly positive correlation was present between Zn and Cr. These positive correlations indicate that the sources of the tested metals within the bees’ nectar-food matrices may have a similar origin. These positive correlations show that the metals are closely associated, thus suggesting their common origin in the bee and honey matrices.

### 3.3. Health risk assessment

The value of EDI, THQ, HI, and TCR of trace metals for male and female adults were computed in some selected districts from Bench Shako Zone throughout the honey consumption pathway. The concentration of Pb is below the detection limits of the method, and therefore the health risk assessment of this trace metal was not computed.

As shown in Table 7, the EDI values of trace metals in honey for male adults was as Zn (2.67E-04) > Cu(1.51E-04) > Mn (6.15E-05) > Cr (2.87E-05) > Cd (1.39E-06) from Sheko district and the order was also in the same manner for female adults. The EDI values from Guraferda and Debub Bench for both male and female adults were shown in a similar order as reported in Sheko. However, the order of EDI for male adults from Mizon Aman Town was Cu (1.70E-04) > Zn (1.46E-04) > Mn (8.04E-05) > Cr (2.05E-05) > Cd (3.11E-06) and its values for female adults was in similar order. The EDI values of Mn obtained from Guraferda were higher than the other trace metals, and its value was lower in the Sheko district for honey consumption by male and female adults. Among all trace metals, the values of EDI of Cd were significantly lower than other trace metals through consumption of honey by male and female adults through consumed honey illustrated in (Table 7). The EDI values (mg/kg) of the investigated trace metals were less than the maximum tolerable daily intake (MTDI) of Cr (0.2), Cu(3.0), Mn (5.0), Cd (0.07), and Zn (60.0) [20, 21].
The EDS values of Zn (8.31E-07), Cr (7.05E-08), and Cd (6.67E-08) previously reported from Iran [4] were lower than the present study for the exposed adult population through honey consumption.

The values of THQ presented in Table 7 revealed that THQ values ranged from 4.87E-04 to 1.18E-03 for Zn, 3.78E-03 to 5.18E-03 for Cu, 4.39E-04 to 7.86E-04 for Mn, 9.02E-04 to 9.71E-03 for Cd, and 7.33E-03 to 1.62E-02 for Cr, respectively, and for targeted male and female adults. The values of THQ of trace metals were lower than 1, indicating that trace metal intake through consumption of honey would have no significant health risks in the study areas in both male and female adults. Moreover, the THQ values for all trace metals were comparatively higher in females than males because of the differences in the average BW of the candidates. Therefore the risk assessments based on EDI, THQ, HI, and TCR were calculated separately for adult male and female individuals.

Table 7 shows the TCR and $\sum TCR$ values for consumption of Cd and Cr in honey samples. The TCR values of Cd ranged from 3.43E-07 to 1.09E-06 and 3.69E-07 to 1.27E-06 for male and female adults, respectively, and of Cr was ranged from 1.03E-2.26E-05 and 1.10E-05 to 2.43E-05 for male and female adults, respectively, while the $\sum TCR$ values of Cd and Cr for male and female adults ranged from 1.15E-05 to 2.37E-05 and 1.23E-05 to 2.55E-05, respectively. According to the New York State Department of Health Center for Environmental Health [39, 40], the TCR category is described as if TCR $< 1.0E-06$ is low; $1.0E-06$ to 1.0E-03 is moderate; $1.0E-03$ to 1.0E-01 is high, and $> 1.0E-01$ is very high. The carcinogenic risk is an estimate of the probability that a person will develop cancer over their estimated 55-year lifespan. Risk values greater than 1.0E-04 are considered intolerable, risks smaller than 1.0E-06 do not result in any adverse health effects, and risks between 1.0E-04 and 1.0E-06 are generally considered in the safe range [6, 14]. The TCR value of Cd for male and female adults from Sheko, Guraferda, and Debub Bench were found in the range of 3.34E-07 to 7.77E-07 were less than unity, suggesting that the consumption of honey does not pose any potential health risks to the exposed population [3, 6].

In conclusion, the present study aimed to investigate the levels of trace metals and human health risk assessment of honey obtained from Southwest Ethiopia using AAS. The optimized method was applied for trace metal analysis in honey and it was validated through the recovery and comparative analysis conducted on the data for honey production efficiency: empirical evidence from Southern Ethiopia, Agric. Food Secur. 9 (1) (2020) 1–13.

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