Determination of some heavy metals in honey from different regions of Ethiopia

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Abstract: Honey is an important bee product of greater nutritional value. Ethiopia is one of the main producers of it. Honey is known to accumulate trace metals which are essential as well as toxic. Moreover, as bees collect nectar from flora of nearly in an area of around 100 km radius, the level of toxic metals in honey is an important indicator for environmental pollution by toxic metals. Thus, in this study, the qualities of 12 Ethiopian multi-floral honey samples were evaluated in terms of common physicochemical parameters, namely, moisture content, pH, free acidity, lactonic acidity, and total acidity. The values found were moisture content from 14.23 to 19.2%; pH from 3.4 to 4.8; free acidity from 30.6 to 97.6 meq Kg$^{-1}$; lactonic acidity from 8.06 to 14.4 meqKg$^{-1}$, and Total acidity from 42.96 up to 107.4 meqKg$^{-1}$. The analyzed samples showed proper maturity, absence of undesirable fermentation, and were in agreement with standard values (Codex Alimentarius).

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PUBLIC INTEREST STATEMENT
Honey is an important nutritional food consumed all over the world. Ethiopia is one of the main producers of this food. The chemical composition of honey may vary depending on floral source, seasonal and environmental factors.

Although Ethiopia had a wide geographical variation, plant flora, and environmental diversity hence thereof different agro-climatic conditions for honey production, the chemical composition of honey in different areas is not well investigated. Hence, this article studies the levels of trace metals (minerals in which some are essential to humans but some are non-essential and toxic) and physicochemical parameters (that indicate Honey quality) in Ethiopian honey selecting representative samples throughout the country. Based on the study consumers, nutritionists, and health professionals can judge the honey quality in Ethiopia with respect to the parameters investigated. The results found are within the international guidelines set up limits and thus the honey from Ethiopia can be determined as safe to human consumption with respect to the studied parameters.
The concentrations of trace heavy metals (Cd, Cr, Cu, Mn, Ni, Pb, and Zn) in 12 multi-floral honey samples were also evaluated using flame absorption spectrometry after wet digestion. The accuracy of the method was assessed by spiking honey samples with known amounts of standard metals and examining recovery. The contents of trace metals in honey samples were found to be in the range of Non-Detectable (ND)-0.017 μg⁻¹, ND-0.15 μg⁻¹, 0.02–1.15 μg⁻¹, ND-7.29 μg⁻¹, ND, ND-2.53 μg⁻¹ and 9.96–16.03 μg⁻¹ for Cd, Cr, Cu, Mn, Ni, Pb, and Zn, respectively. Results obtained are in agreement with a report in the literatures and with codex limits and thus they did not pose as such any health risk.

**Subjects:** Food Additives & Ingredients; Food Chemistry; Food Laws & Regulations

**Keywords:** honey; physicochemical parameters; Ethiopia; wet digestion; atomic absorption spectrometry; trace elements

1. **Introduction**

Honey is a natural sweet substance produced by bees (*Apis mellifera*) from the nectar of different plants, as well as from honeydew (Codex Alimentarius Commission Standards, 2002). It is a highly hygroscopic and very concentrated aqueous solution of sugars with complex matrix. Its composition is variable owning to the differences in plant species, environmental conditions, climate, and beekeeper contribution (De Rodriguez et al., 2004; Küçük et al., 2007). Honey contains different sugars, organic acids, proteins, enzymes, hormones, yeast, vitamins, minerals, and heavy metals (Kujawski & Namieśnik, 2008; Wang & Li, 2011).

Honey is known to accumulate trace metals, cadmium, nickel, and lead which are known as toxic metals, and other essential metals like zinc, copper, manganese, and chromium which are important for health and development of human being in a certain limit (Hernández et al., 2005; Pohl, 2009). However, excess intake of such elements still can cause chronic toxicity (Ashenef, 2014).

It is a useful bio-monitoring mechanism for information related to the environment where the bees live, since honey bees readily fly up to 4 km in all directions from their apiary and thus have access to an area of about 50 km². Thus, the bees come in contact not only with air but also with soil and water, the concentration of heavy metals in honey reflects their amount in the whole environment. Therefore, honey has been recognized as a biological indicator of environmental pollution (Silici et al., 2008).

The mineral and heavy metal contents of honey have been the subject of many studies using different analytical methods including Atomic absorption spectroscopy (FAAS, GFAAS) (Tuzen et al., 2007); inductively coupled plasma-optical emission and mass spectrometry (ICP-AES and ICP-MS) (Aghamirlou et al., 2015; Kılıç Altun et al., 2017); capillary zone electrophoresis (Suárez-Luque et al., 2005); Potentiometric stripping analysis (Munoz & Palmero, 2006); particle-induced X-ray emission (PIXE) (Braziewicz et al., 2002); total reflection X-ray fluorescence (TXRF) (Khuder et al., 2010); ion chromatography (Buldini et al., 2001), and isotope dilution inductively coupled plasma mass spectrometry with direct injection nebulization (ID-ICP-MS) (Packer & Giné, 2001). The analytical methods employed as well as the values found for physicochemical parameters and metal content of Honey around the globe had been recently extensively reviewed by Solayman et al. (2016).

Ethiopia is home to some of the most diverse flora and fauna in Africa and owing to its varied ecological, climatic conditions, it has a big honey production potential. Beekeeping is one of the oldest farming practices in the country. However, the products obtained from this subsector are still low as compared to the potential of the country (Deffar, 1998). Ethiopia, having the highest...
number of bee colonies and surplus honey with many sources of flora, is the leading producer of honey (41 million kilograms of honey/annum) and beeswax in Africa. According to the central statistics agency of Ethiopia 2009/2010 report (the latest available full census in Ethiopia), the total honey production was estimated to be 41 million kilograms and from which Southern Nation Nationality and people of Ethiopia (SNNP) (11,794,672 kg), Oromia (15,825,245 kg), Amhara (7,453,349 kg) and Tigray (3,203,088 kg) account the major portion of honey produced. The report enumerates the number of hives, frequency of honey production, and quantity of honey production per harvest (CSA, 2009/10).

Physicochemical and mineral contents of a specific region (Tigray) and other regions of Ethiopian honey have been studied by few researchers (Belay et al., 2013; Gebremedhin et al., 2013; Nigussie et al., 2012; Liben et al., 2018; Teka, 2018; Yohannes et al., 2018). Both studies perform the investigation either in a specific locality honey or commercial products in a specific city. There is no literature that studied and performed assessment that included the top honey-producing regions of Ethiopian honey with wider geographical coverage at a time. Therefore, the aim of this study was to assess the levels of trace metals in different regions of Ethiopian honey and to characterize it with respect to some physicochemical parameters. Such information is important to beekeepers, as it helps them to avoid possible contamination during honey processing, and to consumers about the quality of their honey thus its safety upon consumption.

2. Materials and methods

2.1. Sampling

The study was conducted on 12 multi-floral honey samples produced from different regions (12 locations) of Ethiopia (Figure 1).

The samples were obtained by taking randomly four samples from commercial honey traders and/or beekeepers from each zone and/or district by the researchers. Then, the samples were mixed, homogenized, and preserved in plastic containers. The honey samples were immediately transferred to the laboratory and kept at 4–5°C until analysis.
2.2. Apparatus
Atomic absorption spectrophotometer: Flame & graphite furnace system (PG990, United Kingdom) equipment fitted with appropriate hollow cathode lamps was used for metal analysis. The operating parameters for the elements determined were set as recommended by the manufacturer (Table 1). Flame measurement was done at a 10 cm long slot-burner head. A lamp and an air-acetylene flame were used. Analytical balances (Mettler Toledo, Switzerland), Abbe refractometer (ATAGO, UK), pH meter (JENWAY 3510, UK) were also used.

2.3. Reagent
HNO₃, H₂O₂, NaOH, KHP, all which are analytical grade from BDH, England, and stock solutions with 1000 mg/L concentration of Cd, Pb, Cu, Mn, Ni, Cr, and Zn, all from Inorganic Ventures, USA were utilized.

2.4. Methods

2.4.1. Physicochemical analysis
The samples of honey were analyzed according to the AOAC methods (Association of Analytical Communities [AOAC], 2000) in order to determine moisture content and acidity (free, lactone, and total). Moisture in honey was determined with the use of a refractometer, reading performed at 20°C and corresponding % moisture obtained from the table (AOAC, 2000). Acidity was determined as follows: briefly, 10 g of honey was dissolved in 75 ml of carbon dioxide-free water in a 250 ml beaker, and stirred with a magnetic stirrer. After homogenization was achieved, the pH electrode was immersed and then the pH was measured. Starting from this pH value, the sample solution was titrated against a standardized 0.05 M NaOH till pH 8.5, and addition of 0.05 M NaOH was stopped at pH 8.50 (free acidity), immediately a volume of 10 ml 0.05 M NaOH was added and, without delay, back-titrated with 0.05 M HCl to pH 8.30 (lactonic acidity). Total acidity results were obtained by adding free and lactone acidities (AOAC, 2000).

2.4.2. Metal analysis
The procedure described by (Tuzen et al., 2007) was followed for the digestion of honey samples. In this method, 1 g of honey sample, mixed and homogenized, was weighed and placed in a conical flask. Eight (8) ml of concentrated nitric acid and 4 ml of hydrogen peroxide were added to the flask containing the sample. Then, the sample mixture was placed on the water bath and heated for 4 h to dryness. The flask was removed from the water bath and cooled to room temperature. To the cooled sample, de-ionized water was added to dissolve the dried mass, and the content was filtered in a 10 ml volumetric flask with whatman no. 42 filter paper. Subsequently, the solution was made up to volume using de-ionized water. Similarly, reagent blank was prepared by taking a mixture of the reagents (concentrated nitric acid and hydrogen peroxide) and treating it in the same manner as the sample.

| Parameters | Cu | Ni | Zn | Mn | Cr | Pb | Cd |
|------------|----|----|----|----|----|----|----|
| Wavelength (nm) | 324.7 | 232.0 | 213.9 | 279.5 | 357.9 | 283.3 | 228.8 |
| Spectral interval width (nm) | 0.4 | 0.2 | 0.4 | 0.2 | 0.4 | 0.4 | 0.4 |
| Supply current of discharge lamp (mA) | 3.0 | 2.0 | 3.0 | 2.0 | 4.0 | 2.0 | 2.0 |
| Background correction | No | D₂ | D₂ | D₂ | No | D₂ | D₂ |
The content of the flask was then analyzed for the level of the selected heavy metals (Cd, Cr, Cu, Mn, Ni, Pb, and Zn) if any using atomic absorption spectrophotometer: Flame & graphite furnace system (PG990, United Kingdom). The parameters for the instrument were set per the company recommendation as shown above in Table 1. The instrument was calibrated by using standard solutions of studied metals. The whole procedure was done in triplicate.

3. Results and discussion

3.1. Physicochemical parameters

Results for the studied physicochemical parameters of honey from different regions of Ethiopia are summarized in Table 2.

The moisture content found was between 14.23% and 19.2%. Moisture level in honey is a quality criterion that determines the capability of honey to remain stable and to resist spoilage by yeast fermentation (Bogdanov et al., 2002). It is also an important character to determine its stability (the shelf life of honey) during storage (Nanda et al., 2003). None of the honey samples exceeded the permitted limit established by the European Community Directive (not more than 20%) (Codex Alimentarius Commission Standards, 2002). The values found correspond to mature honey, and it indicates that the beekeepers had followed the proper time of extraction. As moisture content is dependent on botanical origin, harvest season, and proper time of extraction, there was a significant variation between honeys from different regions. Similar results were obtained in previous studies in Turkey, 15.36–18.5% (Özcan et al., 2006); Syria, 13.2–19.4%, (Khuder et al., 2010); New Zealand, 15.7–18.0%, (Vanhanen et al., 2011); Saudi Arabia, 14.40–15.95%, (Osman et al., 2007); Greek, 13.0–18.9% (Lazaridou et al., 2004).

The pH values of all honey samples collected from the different regions are in the acidic range (3.4–4.8). The lowest pH was found for honey samples collected from Benchi-Majji, and the highest pH from Debere Markos. Except the pH of honey collected from Benchi-Majji, all the mean pH values were within permitted limit established by the European Community Directive (3.5–5.5). The mean pH value of Ethiopian honey was similar to Turkey, 3.94 (Kahraman et al., 2010), Spain, 3.56–4.79.

Table 2. Statistical analysis of some studied physicochemical parameters of Ethiopian multi-floral honey

| Sample     | pH     | Moisture content (%) | Free acidity (meqKg⁻¹) | Lactonic acidity (meqKg⁻¹) | Total acidity (meqKg⁻¹) |
|------------|--------|----------------------|------------------------|----------------------------|-------------------------|
| Adigrat    | 3.8 ± 0.05<sup>a</sup> | 17.16 ± 0.15<sup>b</sup> | 33.3 ± 2.88<sup>cd</sup> | 12.76 ± 0.60<sup>d</sup> | 46.06 ± 2.35<sup>d</sup> |
| ArsiRobe   | 3.6 ± 0.10<sup>a</sup>  | 18.82 ± 0.03<sup>a</sup> | 63.3 ± 2.31<sup>cd</sup> | 9.33 ± 1.21<sup>d</sup>  | 72.33 ± 3.44<sup>d</sup> |
| BenchiMajji| 3.4 ± 0.10<sup>a</sup>  | 14.23 ± 0.05<sup>a</sup> | 97.6 ± 6.80<sup>d</sup>  | 9.8 ± 0.6<sup>d</sup>    | 107.4 ± 6.23<sup>d</sup>|
| Bure       | 4.5 ± 0.05<sup>a</sup>  | 14.56 ± 0.05<sup>a</sup> | 42.6 ± 2.51<sup>cd</sup> | 8.43 ± 1.88<sup>d</sup>  | 51.03 ± 3.12<sup>d</sup> |
| Bonga      | 4.1 ± 0.05<sup>b</sup>  | 15.11 ± 0.10<sup>d</sup> | 36.0 ± 1.00<sup>d</sup>  | 9.9 ± 0.1<sup>d</sup>    | 45.9 ± 0.90<sup>d</sup> |
| Dawero     | 4.7 ± 0.11<sup>c</sup>  | 16.57 ± 0.39<sup>c</sup> | 40.6 ± 3.05<sup>c</sup>  | 8.06 ± 0.11<sup>c</sup>  | 48.66 ± 3.03<sup>c</sup> |
| D/Markos   | 4.8 ± 0.00<sup>c</sup>  | 19.2 ± 0.01<sup>c</sup>  | 41.33 ± 2.31<sup>c</sup> | 9.73 ± 1.60<sup>c</sup>  | 51.06 ± 2.71<sup>c</sup> |
| Erob       | 3.8 ± 0.05<sup>b</sup>  | 16.39 ± 0.03<sup>b</sup> | 31.0 ± 3.46<sup>cd</sup> | 14.4 ± 0.75<sup>b</sup>  | 45.4 ± 4.09<sup>b</sup>  |
| Ginchi     | 4.2 ± 0.05<sup>b</sup>  | 17.64 ± 0.07<sup>d</sup> | 44.6 ± 0.57<sup>cd</sup> | 11.0 ± 0.3<sup>d</sup>   | 55.6 ± 0.85<sup>cd</sup> |
| Jimma      | 4.1 ± 0.00<sup>c</sup>  | 14.47 ± 0.03<sup>c</sup> | 55.0 ± 5.00<sup>cd</sup> | 10.26 ± 0.68<sup>c</sup> | 65.26 ± 4.52<sup>cd</sup>|
| Hawzene    | 3.8 ± 0.00<sup>c</sup>  | 18.38 ± 0.02<sup>c</sup> | 30.6 ± 1.52<sup>cd</sup> | 12.36 ± 0.57<sup>cd</sup> | 42.96 ± 1.10<sup>cd</sup> |
| Tillili    | 4.5 ± 0.10<sup>b</sup>  | 17.32 ± 0.03<sup>d</sup> | 33.3 ± 1.52<sup>cd</sup> | 10.86 ± 2.11<sup>d</sup> | 44.19 ± 2.72<sup>d</sup> |
| Codex<sup>a</sup> | 3.5-5.5 | <20 | <50 | - | - |

NB: Mean in a column with different letters (a-g) are significantly different (P < 0.05). Codex Alimentarius Commission Standards (2002)
The acidity of honey is due to the presence of organic acids, particularly the gluconic acid, in equilibrium with their lactones or esters and also due to inorganic ions such as phosphate and chloride (Ouchemoukh et al., 2007). The mean values of free acidity in studied samples had exhibited the minimum value as 30.6 meqKg⁻¹ for Hawzene and the maximum free acidity value for Benchi-Maji (97.6 meqKg⁻¹). The mean free acidity of Ethiopian honey (45.81 meqKg⁻¹) was found to be the highest compared to the reported values from countries such as Turkey, 22.8 meqKg⁻¹ (Kahraman et al., 2010), Spain, 17.59–39.81 meqKg⁻¹ (Terrab et al., 2004), Slovenia, 11.67–17.11 meqKg⁻¹ (Krapf et al., 2010), and India, 14.57–32.65 meqKg⁻¹ (Nanda et al., 2003). Lactonic acidity, considered as the acidity reserve when the honeys become alkaline ranges from 8.06 meqKg⁻¹ in the case of Dawero to 14.4 meqKg⁻¹ for Erob. While total acidity was between 42.96 meqKg⁻¹ for Hawzene and 107.4 meqKg⁻¹ for Benchi-Maji. Twenty-five (25%) of honey samples exceeded the maximum limit of free acidity established by the European Community Regulation.

3.2. Determination of studied metals

The accuracy of the method was assessed by spiking honey samples with known amounts of standard metals, and examining recovery. Table 3 shows the recovery and method detection limit (MDL) results for the studied metals. The results show the validity of the employed methods and a good repeatability for the analysis of honey samples. Reading of control solution between three consecutive samples showed the instrument’s precision (97.5%–102.1%). In addition, to evaluate the method detection limit (MDL), six blank solutions (all treatment procedures for the analysis of sample were done except the inclusion of the sample itself) were digested in triplicate and the absorbance was recorded. Then, MDL was calculated as three times the standard deviation of the blank (3σ blank).

Metals were measured in the honey samples utilizing the FAAS instrument by external calibration method. The concentration of metals in honey samples varied in the order of Zn > Mn > Cr > Pb > Cu > Cd > Ni (Table 4).

Cadmium was detected in three honey samples ranging from 0.017 μg g⁻¹–0.035 μg g⁻¹, the highest being from Bure and the lowest from Arsi. The high concentration of Cd in Bure might be attributed to the high traffic, poor sewage system, use of fertilizers, and use of animal waste product (Cow/Ox dung smoke) for the collection of honey from hives. The mean level of Cd in present studied honey samples (0.027 μg g⁻¹) did not exceed the limit established by Codex Alimentarius Commission, 0.05 μg g⁻¹ (Codex Alimentarius Commission Standards, 2002), Turkish codex 0.03 μg g⁻¹ (Leblebici & Aksoy, 2008), Indian regulations, 1.5 μg g⁻¹ (Nanda et al., 2003), Australia Food standard code (0.05 μg g⁻¹), and Macedonia legislation, 0.03 μg g⁻¹ (Staniškienė et al., 2006). The mean value of cadmium from the present study (ND–0.017 μg g⁻¹) was comparable with reports from Egypt, 0.01–0.5 μg g⁻¹ (Rashed & Soltan, 2004), New Zealand, 0.01–0.45 μg g⁻¹ (Vanhanen et al., 2011) and Turkey Central Anatolia, 0.09–0.24 μg g⁻¹ (Leblebici & Aksoy, 2008).

Chromium level was the highest in honey samples obtained from Tilili, 6.66 μg g⁻¹ followed by Bure, 5.89 μg g⁻¹, Adigrat, 5.89 μg g⁻¹, Erob, 0.16 μg g⁻¹ and Debre Markos, 0.15 μg g⁻¹. Contact with stainless steel surfaces during the harvesting processing and/or preparation of honey for the market can generate high chromium content, due to the corrosive effect of honey acidity (Tuzen et al., 2007). The mean concentration of Cr in Ethiopian honey ranged from, ND–6.66 μg g⁻¹, which was lower than values obtained for honey samples collected from Turkey, 2.4–37.9 μg g⁻¹ (Tuzen et al., 2007) and Slovenia, 0.11–33.8 μg g⁻¹ (Golob et al., 2005) but was much higher than values reported in Chile, 0.03–1.92 μg g⁻¹ (Fredes & Montenegro, 2006), Italy (Siena), 2.0–54 μg g⁻¹, (Pisani et al., 2008), Nigeria, 0.32 μg g⁻¹ (Achudume & Nwafor, 2010).
| Metals | Concentration found (mg Kg⁻¹) | Concentration after spiking (mg Kg⁻¹) | Recovery (%) | MDL (mg L⁻¹) | IDL (mg L⁻¹) |
|--------|------------------------------|-------------------------------------|--------------|--------------|--------------|
| Cd     | 0.030                        | 0.044                               | 93.3         | 0.0159       | 0.0028       |
| Cr     | 6.66                         | 10.04                               | 101.5        | 0.0718       | 0.005        |
| Cu     | 1.15                         | 1.71                                | 97.4         | 0.0041       | 0.004        |
| Mn     | 7.29                         | 11.16                               | 106.3        | 0.0375       | 0.008        |
| Ni     | ND                           | 4.12                                | 102.7        | 0.0169       | 0.002        |
| Pb     | 2.53                         | 3.66                                | 90.2         | 0.2258       | 0.03         |
| Zn     | 14.62                        | 22.63                               | 105.5        | 0.2430       | 0.003        |

IDL: instrumental detection limit, MDL: method detection limit.
ND: not detected.
Table 4. Trace metal contents in wet digested honey samples from different regions of Ethiopia (mean ± SD, n = 3), wet mass

| Sample      | Cd (μg g⁻¹) | Pb (μg g⁻¹) | Zn (μg g⁻¹) | Cu (μg g⁻¹) | Cr (μg g⁻¹) | Mn (μg g⁻¹) | Ni (μg g⁻¹) |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Benchi-Maji | ND          | ND          | 16.03 ± 0.29 | 0.45 ± 0.04 | ND          | ND          | ND          |
| Bonga       | ND          | ND          | 11.62 ± 0.63 | 0.026 ± 0.04 | ND          | ND          | ND          |
| Dawero      | ND          | ND          | 14.3 ± 0.16  | 0.21 ± 0.04  | ND          | ND          | ND          |
| Ginchii     | ND          | 1.64 ± 0.08  | 10.43 ± 0.64 | 0.24 ± 0.00  | ND          | ND          | ND          |
| Arsirabe    | 0.017 ± 0.0025 | ND          | 13.63 ± 1.09 | 0.21 ± 0.01  | ND          | ND          | ND          |
| Jimma       | 0.03 ± 0.0015 | 1.23 ± 0.07  | 15.17 ± 0.33 | 0.08 ± 0.00  | ND          | ND          | ND          |
| Adigrat     | ND          | 0.23 ± 0.12  | 12.77 ± 0.40 | 0.10 ± 0.09  | 5.89 ± 0.44  | ND          | ND          |
| Erob        | ND          | ND          | 12.58 ± 0.39 | 0.16 ± 0.00  | 0.16 ± 0.00  | ND          | ND          |
| Hawzene     | ND          | ND          | 10.65 ± 0.38 | ND          | ND          | ND          | ND          |
| Tilili      | ND          | ND          | 14.02 ± 0.31 | 0.32 ± 0.00  | 6.66 ± 0.44  | 7.29 ± 0.04  | ND          |
| DiMarkos    | ND          | 2.53 ± 0.03  | 9.96 ± 0.46  | 1.15 ± 0.04  | 0.15 ± 0.00  | 0.36 ± 0.00  | ND          |
| Bure        | 0.035 ± 0.005 | ND          | 14.62 ± 0.13 | 0.02 ± 0.004 | 5.89 ± 0.00 | 0.7 ± 0.07 | ND          |
| Codex       | 0.05        | 0.3         | 5           | 5           | -           | 0.36 ± 0.00 | ND          |
| India       | 1.5         | 2.5         | -           | 30          | -           | -           | 50          |
| Australia   | 0.05        | 1.5         | -           | 10          | -           | -           | -           |

ND: Concentration of metals below method detection limit (< MDL). Mean in a column with different letters (a-e) are significantly different (P < 0.05) NB: Codex Alimentarius Commission Standards (2002)⁵, India regulation, Nanda et al. (2003)⁶, Australia Food standard code.⁷

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Copper was one of the heavy metals detected in all honey samples analyzed except honey samples collected from Hawzene. The lowest and the highest copper concentrations were 0.02 μg g\(^{-1}\) in the honey sample from Bure and 1.15 μg g\(^{-1}\) in honey sample from Debre Markos. The mean Cu levels in studied samples did not exceed the limit established by Codex Alimentarius Commission, 5 μg g\(^{-1}\) (Codex Alimentarius Commission Standards, 2002), Indian regulations (30 μg g\(^{-1}\)), and Australia Food standard code (10 μg g\(^{-1}\)). The level of copper observed in the present study (ND-1.15 μg g\(^{-1}\)) was in agreement with results reported from Chile, 0.06–2.00 μg g\(^{-1}\) (Fredes & Montenegro, 2006), Czech, 0.11–0.88 μg g\(^{-1}\) (Lachman et al., 2007), Turkey black sea region, 9.75–35.8 μg g\(^{-1}\) (Silici et al., 2008), and Turkey middle Anatolia, 0.25–1.10 μg g\(^{-1}\) (Tuzen et al., 2007).

Only samples originated from Amhara regions (Bure, Debre Markos, and Tiliili) showed the presence of manganese. It was found that the maximum concentration of Mn was 7.29 μg g\(^{-1}\) from Tiliili, followed by Bure, 0.7 μg g\(^{-1}\) and Debre Markos, 0.36 μg g\(^{-1}\). It was observed that the value obtained was comparable with results reported by Madejczyk and Baralkiewicz, 2008 from Poland, 0.02–7.37 μg g\(^{-1}\), Almeida-Silva et al. (2011), from Portugal, 0.83 μg g\(^{-1}\), Suárez-Luque et al. (2005), from Spain, 2.3–5.0 μg g\(^{-1}\), and Tuzen et al. (2007), from Turkey, 0.32–4.56 μg g\(^{-1}\).

Lead was detected in 33.3% (4/12) of the analyzed samples ranging from 0.23 μg g\(^{-1}\) to 2.53 μg g\(^{-1}\). The lowest and the highest lead concentrations were 0.23 μg g\(^{-1}\), in the honey sample from Adigrat, and 2.53 μg g\(^{-1}\) in the honey sample from Debre Markos. The variation of the mean level of lead may be attributed to the presence of highways, presence of metal workshops, house construction tools, and use of extensive fertilizers for the production of crops. The lead level in Ethiopian honey was higher than the mean concentration reported by (Chudzinska, 2010) from Poland, LD-9.20 μg g\(^{-1}\), Turkey, 0.451 μg g\(^{-1}\) (Özcan et al., 2006), Turkey central Anatolia, 0.02–1.50 μg g\(^{-1}\) (Leblebici & Aksoy, 2008), Turkey Black sea region, 1.54–36.7 μg g\(^{-1}\) (Silici et al., 2008), Nigeria, 0.13 μg g\(^{-1}\) (Achudume, 2010), New Zealand, ND-0.0170 μg g\(^{-1}\) (Vanhonen et al., 2011), Lithuania, 2.9–2.21 μg g\(^{-1}\) (Staniškienė et al., 2006), and Italy sienna, 28.2–304 μg g\(^{-1}\) (Pisani et al., 2008).

In this study, Zn was the only metal detected in all analyzed honey samples. The maximum concentration of Zn was found in a honey sample collected from Benchi-Maji, 16.03 μg g\(^{-1}\), and the lowest was from Debre Markos, 9.96 μg g\(^{-1}\). Usually, the use of galvanized containers is the most
Table 5. Trace metal Concentrations, amount in one and two spoons of honey, and the contribution of two spoons of ingested honey to (Daily Dose Allowance) D.D.A

| Metal | Sample     | Concentration | Amount in one spoon (μg) | Amount in two spoon (μg) | DDA  | Contribution to DDA (%) |
|-------|------------|---------------|--------------------------|--------------------------|------|-------------------------|
| Cu    | BenchiMajji| 0.45 ± 0.04   | 9                        | 18                       | 3 mg | 0.6                     |
|       | Bonga      | 0.026 ± 0.04  | 0.52                     | 1.04                     |      | 0.035                   |
|       | Dawero     | 0.21 ± 0.04   | 4.2                      | 8.4                      |      | 0.28                    |
|       | Ginchi     | 0.24 ± 0.00   | 4.8                      | 9.6                      |      | 0.32                    |
|       | Arsi Robe  | 0.21 ± 0.01   | 4.2                      | 8.4                      |      | 0.28                    |
|       | Jimma      | 0.08 ± 0.00   | 1.6                      | 3.2                      |      | 0.11                    |
|       | Adigrat    | 0.10 ± 0.09   | 2                        | 4                        |      | 0.13                    |
|       | Erab       | 0.16 ± 0.00   | 3.2                      | 6.4                      |      | 0.21                    |
|       | Tili       | 0.32 ± 0.00   | 6.4                      | 12.8                     |      | 0.43                    |
|       | DMarkos    | 1.15 ± 0.04   | 23                       | 46                       |      | 1.53                    |
|       | Bure       | 0.02 ± 0.004  | 0.4                      | 0.8                      |      | 0.03                    |
| Mn    | Tili       | 7.29 ± 0.04   | 145.8                    | 291.6                    | 4 mg | 7.29                    |
|       | DMarkos    | 0.36 ± 0.00   | 7.2                      | 14.4                     |      | 0.36                    |
|       | Bure       | 0.7 ± 0.07    | 14                       | 28                       |      | 0.7                     |
| Zn    | BenchiMajji| 16.03 ± 0.29  | 320.6                    | 641.2                    | 15 mg| 4.27                    |
|       | Bonga      | 11.62 ± 0.63  | 232.4                    | 464.8                    |      | 3.10                    |
|       | Dawero     | 14.3 ± 0.16   | 286                      | 572                      |      | 3.81                    |
|       | Ginchi     | 10.43 ± 0.64  | 208.6                    | 417.2                    |      | 2.78                    |
|       | Arsi Robe  | 13.63 ± 1.09  | 272.6                    | 545.2                    |      | 3.63                    |
|       | Jimma      | 15.17 ± 0.33  | 303.4                    | 606.8                    |      | 4.05                    |
|       | Adigrat    | 12.77 ± 0.40  | 255.4                    | 510.8                    |      | 3.41                    |
|       | Erab       | 12.58 ± 0.39  | 251.6                    | 503.2                    |      | 3.35                    |
|       | Hawzene    | 10.65 ± 0.38  | 213                      | 426                      |      | 2.84                    |
|       | Tili       | 14.02 ± 0.31  | 280.4                    | 560.8                    |      | 3.74                    |
|       | DMarkos    | 9.96 ± 0.4    | 199.2                    | 398.4                    |      | 2.66                    |
|       | Bure       | 14.62 ± 0.13  | 292.4                    | 584.8                    |      | 3.90                    |
prominent source of contamination of honey besides the soil and flora differences of forage area (Tuzen et al., 2007). All the honey samples analyzed for the presence of Zn exceeded the maximum permitted level established by Codex Alimentarius Commission, 5 μg g⁻¹ (Codex Alimentarius Commission Standards, 2002). The mean concentration of Zn reported from Poland, LD −39.7 μg g⁻¹ (Chudzinska & Baralikiewicz, 2010), India, 2.55–16.77 μg g⁻¹ (Nanda et al., 2003), Egypt, 5.00–9.3 μg g⁻¹ (Rashed & Soltan, 2004), Canary Island, 0.18–19.1 μg g⁻¹ (Hernández et al., 2005), and Slovenia, 0.55–11.2 μg g⁻¹ (Golob et al., 2005) was comparable with the mean concentration of Zn in samples of honey collected from Ethiopia (9.96–16.03 μg g⁻¹)

As shown in Figure 2, honey samples collected from SNNPRs had the highest Zn level, followed by Oromia, Amhara, and Tigray. Cu was found in the highest amount in samples collected from SNNP followed by Amhara, Oromia, and Tigray. Honey collected from SNNPRs and Oromia did not contain Cr while it was detected in samples from Amhara and Tigray regions. The amount of Cr in samples from Amhara region was observed to be higher than that of the Tigray region. Mn was found in honey samples collected from Amhara region only. Pb was detected in samples from the studied regions except SNNPRs. In addition, it has been shown that samples collected from Oromia and Amhara regions show the presence of Cd but it was not detected in honey samples originating from Tigray and SNNP regions. The difference might be associated with geographical causes, recent man-made activities and climatic conditions seen in this top honey-producing regions.

3.3. Evaluation of the nutritional value of Ethiopian honey
To evaluate the nutritional value, one and two tablespoons of each honey were weighed. Considering the concentration of the elements in the honey samples, the contents of those elements in one and two spoons were calculated and compared to their daily dose allowance (D.D.A.) in the human body (Almeida-Silva et al., 2011). This study tries to compare three studied metals (Cu, Mn, and Zn). Table 5 summarizes the contribution of two spoons of honey on daily dose allowance. Since there is no stated daily consumption of honey in Ethiopia, it is difficult to compare the toxic effect of heavy metals studied with the continuous and immediate ingestion of honey. The ingestion of two tablespoons of Ethiopian honey represents between 0.035% and 7.29% of the D.D.A.

4. Conclusion
Honey from Ethiopia collected from different geographical areas was assessed for physicochemical parameters, namely, moisture content, pH, free acidity, lactic acid, and total acidity. Trace heavy metals (Cd, Cr, Cu, Mn, Ni, Pb, and Zn) levels were also determined. The values found are in agreement with Codex recommendations. However, the study showed a significant difference between samples collected from different regions in terms of studied physicochemical parameters and heavy metals. The variation may be attributed to greatly on geographical factors, distance of bee forage area from roadsides, soil composition, and anthropogenic sources.

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Competing Interests
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111 different types of Egyptian mono-floral and non-floral bee honeys. Journal of Food Composition and Analysis, 17(6), 725–735. https://doi.org/10.1016/j.jfca.2003.10.004
Silici, S., Uluozlu, O. D., Tuzen, M., & Soyak, M. (2008). Assessment of trace element levels in Rhododendron honeys of Black Sea Region, Turkey. Journal of Hazardous Materials, 156(1–3), 612–618. https://doi.org/10.1016/j.jhazmat.2007.12.065
Solayman, M., Islam, M. A., Paul, S., Ali, Y., Khoil, M. I., Alam, N., & Gan, S. H. (2016). Physicochemical properties, minerals, trace elements, and heavy metals in honey of different origins: A comprehensive review. Comprehensive Reviews in Food Science and Food Safety, 15(1), 219–233. https://doi.org/10.1111/1541-4337.12182
Stanisikienė, B., Matusiūvičius, P., & Būdreckienė, R. (2006). Honey as an indicator of environmental pollution. Environmental Research, Engineering & Management, 36(2), 53–68. https://hdl.handle.net/20.500.12512/12302
Suárez-Luque, S., Mato, I., Huidobro, J. F., & Simal-Lozano, J. (2005). Capillary zone electrophoresis method for the simultaneous determination of cations in honey. Journal of Chromatography A, 1083(1–2), 193–198. https://doi.org/10.1016/j.chroma.2005.06.011
Teku, A. E. (2018). Levels of some selected trace and essential elements in honey from selected woredas of sidama zone, southern region, ethiopia. J Agric Sci, 2(1), 12–18. doi: 10.35841/2591-7897.2.1.12-18
Terrab, A., Recamales, A. F., Hernanz, D., & Heredia, F. J. (2006). Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. Food Chemistry, 88(4), 537–542. https://doi.org/10.1016/j.foodchem.2004.01.068
Tuzen, M., Silici, S., Mendli, D., & Soyak, M. (2007). Trace element levels in honeys from different regions of Turkey. Food Chemistry, 103(2), 325–330. https://doi.org/10.1016/j.foodchem.2006.07.053
Vanhanen, L. P., Emmertz, A., & Savage, G. P. (2011). Mineral analysis of mono-floral New Zealand honey. Food Chemistry, 128(1), 236–240. https://doi.org/10.1016/j.foodchem.2011.02.064
Wang, J., & Li, Q. X. (2013). Chemical composition, characterization, and differentiation of honey botanical and geographical origins. In S. Taylor (Ed.), Advances in food and nutrition research (Vol. 62, pp. 89–137). Academic Press. https://doi.org/10.1016/B978-0-12-385989-1.00003-X
Yohannes, W., Chandravanshi, B. S., & Moges, G. (2018). Assessment of trace metals and physicochemical parameters of commercially available honey in ethiopia. Chemistry International, 4(2), 91-101. https://bosajournals.com/chemint/images/pdffiles/18-12.pdf
