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*Original Research Article*

# Determination of Trace Metals and Polyphnols in Honey from South and North Wollo Zones, Ethiopia

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## ABSTRACT

Honey is a natural product valued for its nutritional and antioxidant properties, which are influenced by its mineral composition and polyphenolic content. This study aimed to determine the concentrations of selected trace metals (Fe, Ni, Mn, Cu, and Zn) and total polyphenols in honey samples collected from six districts of North Wollo (Wadal, Meket, and Gubalafo) and South Wollo (Ambasel, Delanta, and Tehuledere), Ethiopia. The concentrations of metals were determined by using flame atomic absorption spectroscopy after acid digestion of the honey samples. Total polyphenol contents were determined using the Folin–Ciocalteu method with UV–Vis spectroscopy and results expressed as milligrams of gallic acid equivalents per kilogram (mg GAE/kg). The concentrations of Fe, Ni, Mn, Cu, and Zn in the honey samples ranged from 24.2–85.8 mg/kg, 5.6–15.1 mg/kg, 14.9–25.8 mg/kg, 5.1–16.6 mg/kg, and 12.2–53.5 mg/kg, respectively. The limits of detection (LOD) for Fe, Ni, Mn, Cu, and Zn were 4.2, 1.2, 2.3, 1.4, and 2.1 mg/kg, respectively. Except for samples from Wadal, Fe was the most abundant metal followed by Zn. Method accuracy was evaluated using spiked honey samples, and the mean percentage recoveries ranged from 83.9% to 112.5%, indicating acceptable analytical performance. The polyphenol content of the honey samples ranged from 519.8 to 1221.7 mg GAE/kg. The mean total polyphenol content was higher in honey from North Wollo (893.5 mg GAE/kg) than from South Wollo (707.3 mg GAE/kg). The results indicate that honey from the study areas contains appreciable levels of essential trace metals and bioactive polyphenols, suggesting its potential nutritional and antioxidant benefits.

**Keywords**: Honey, Trace metals, Polyphenol, Wollo, Ethiopia.

## INTRODUCTION

Polyphenols are naturally occurring organic compounds widely distributed in fruits, vegetables, cereals, and beverages. More than 8,000 phenolic compounds have been identified, including over 4,000 flavonoids (Berhanu, 2014). These compounds are well known for their strong antioxidant properties, which enable them to scavenge free radicals and reduce oxidative

stress in biological systems. Consequently, polyphenols are associated with various health benefits, including anti-inflammatory, antimicrobial, and cardioprotective effects. In honey, polyphenols originate mainly from floral nectar and pollen, and their concentration depends on the botanical and geographical origin of the honey (Alvarez-Suarez et al., 2010). Therefore, the polyphenolic content of honey is often used as an indicator of its antioxidant capacity and nutritional quality.

The mineral composition and polyphenolic content of honey vary considerably depending on several factors such as botanical origin, soil composition, environmental conditions, and beekeeping practices. Trace metals in honey may originate from natural sources such as soil and plant uptake, as well as from anthropogenic sources including agricultural activities, industrial emissions, and environmental pollution (Bogdanov et al., 2007). As a result, the determination of trace metals in honey is important not only for evaluating its nutritional value but also for assessing environmental contamination and food safety.

Honey produced in Ethiopia is widely recognized for its quality and diversity due to the country's rich floral resources and favorable agro-ecological conditions. Ethiopia is one of the largest honey producers in Africa, and honey plays an important role in the national economy as well as in traditional food and medicinal practices. However, despite its importance, limited information is available on the mineral composition and bioactive compounds of honey produced in many parts of the country, including the North and South Wollo zones of the Amhara Region.

Therefore, this study aimed to determine the concentrations of selected trace metals (Fe, Ni, Mn, Cu, and Zn) and the total polyphenol content of honey samples collected from selected districts of North and South Wollo zones. The results of this study provide valuable information on the nutritional quality and antioxidant potential of honey from the study area and may also serve as baseline data for future studies on honey quality and environmental monitoring.

## MATERIALS AND METHODS

### Honey Samples

Honey samples were collected from six districts located in the North and South Wollo Zones of the Amhara Regional State, Ethiopia. The sampling districts included Ambasel, Meket, Wadla, and Gubalafto from North Wollo, and Delanta and Tehuledere from South Wollo. In Ambasel District, samples were collected from Erobit, Wuchalie town, and Golbo. In Meket District, the sampling sites were Dabza, Rasdegen, and Achim. In Wadla District, samples were obtained from Meley, Quana, and Yeneja. In Gubalafto District, honey samples were collected from Geshober, Debot, and Anoy. Similarly, in Delanta District the collection sites were Asmkola, Chewukutir, and Sebelet, while in Tehuledere District the samples were obtained from Libanos, Maryam Debir, and Kekewa.

A total of eighteen honey samples were collected from the six districts. As illustrated in Figure 1, three samples were collected from different farmers in each district and composited to form a representative sample for that district. The sampling was conducted in June 2019 during the active honey harvesting season. The collected honey samples were stored in clean plastic containers, properly labeled, and transported to the laboratory at the University of Gondar for further analysis.

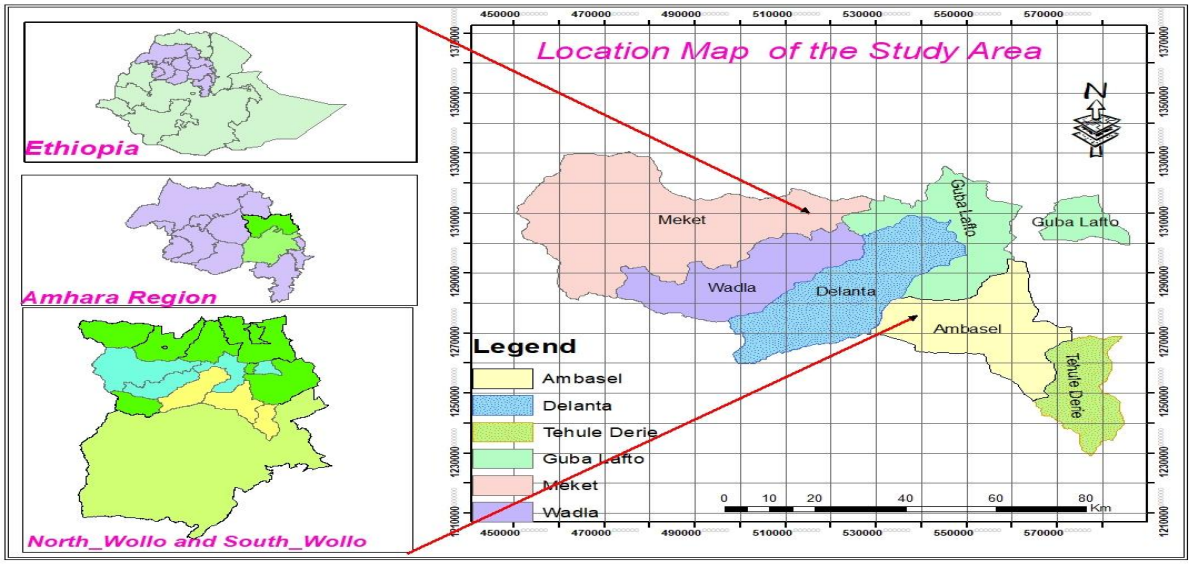


Figure 1. Map of honey sample collection site.

### Instrument and Apparatus

Flam Atomic Absorption Spectrophotometer (Buck Scientific Model, 210 VGP, USA), Conical flasks (100 mL), hotplate (SH3, STERILINELTD.UK), refrigerator (HITACH LR902T, England) and UV-Vis Spectrophotometer (Abron, India) were used in the study.

### Chemicals and Reagents

Gallic acid (Fine Chemicals, Mumbai, India), (70%) HClO4 and (69%) $HNO_3$ (Blulux laboratories, Haryana, India), standard metal solutions (1000 ppm) Zn, Ni, Mn, Cu and Fe (Merck, Germany), Folin-Ciocalteu (Fine Chemicals, Mumbai, India) phenol reagent,7.5% Na2CO3 (Blulux laboratories), methanol (99%,Fine Chemicals, Mumbai India) and distilled water were used in the study.

### Digestion of Honey Sample

Prior to analysis, the honey samples were homogenized in their containers to ensure uniformity. To reduce viscosity and facilitate dissolution of sugars, each sample was heated at 60 °C for 40 minutes. Subsequently, the samples were further homogenized by adding distilled water at a water-to-honey ratio of 1:3 (w/w) and shaking thoroughly to obtain a uniform solution (Doker et al., 2014).

All glassware used in the analysis was thoroughly cleaned by washing with detergent, followed by rinsing with nitric acid and distilled water three times to avoid contamination. The honey samples were then prepared for trace metal determination through acid digestion. Approximately 0.5 g of homogenized honey was accurately weighed and transferred into a 100 mL conical flask. To this, 2 mL of 69% nitric acid ($HNO_3$) and 4 mL of 70% perchloric acid ($HClO_4$) were added. The digestion process was carried out in a fume hood to ensure safety.

The conical flask was covered with a clean watch glass to prevent loss of analytes during heating. The mixture was heated on a hot plate at 150 °C for about 1.5 hours until a clear digest

was obtained. After digestion, the solution was allowed to cool to room temperature. Subsequently, 5 mL of distilled water was added while gently swirling to minimize dissolution of the filter paper by the digestion residue. The solution was then filtered using Whatman filter paper No. 42 into a 25 mL volumetric flask and diluted to the mark with distilled water.

Reagent blanks were prepared using the same volumes of reagents and subjected to the same digestion procedure (temperature and time) as the samples. Three reagent blanks were prepared to account for possible contamination from reagents and glassware. The digested honey solutions were then analyzed for the concentrations of five trace metals (Ni, Cu, Mn, Fe, and Zn) using a flame atomic absorption spectrophotometer (FAAS).

### FAAS Determination of Elements

The concentrations of trace elements in the digested honey samples were determined by using flame atomic absorption spectrophotometer (FAAS) equipped with deuterium arc background corrector and air-acetylene flame at different operating conditions used for FAAS for each of the elements (Table 1).

**Table 1**. Instrumental condition for the determination of metals in honey sample by FAAS.

| Element | Lamp current (mA) | Energy (eV) | Slit width (nm) | Wavelength (nm) |
|---|---|---|---|---|
| Ni | 7.0 | 2.901 | 0.2 | 240.3 |
| Cu | 1.5 | 3.75 | 0.7 | 324.7 |
| Zn | 2.0 | 3.154 | 0.72 | 213.7 |
| Mn | 3.05 | 4.142 | 0.7 | 279.5 |
| Fe | 7.0 | 3.047 | 0.2 | 248.3 |

The atomic absorption spectrometer was calibrated using five point standard solutions, corresponding to each element, in the concentration (mg L−1) range of 0.1–8.1 for Mn, 0.1– 8.1 for Fe, 0.01– 6.25 for Ni, 0.02– 5.12 for Cu and 0.1–8.1 for Zn. The correlation coefficients of the calibration curves were greater than 0.999 for all the analyzed metals (Table 2).

**Table 2**. Calibration equations, correlation coefficients (r2), limits of detection (LOD) and limits of quantitation (LOQ) of the FAAS method.

| Element | Correlation equation | $r^2$ | LOD(mg/kg) | LOQ(mg/kg) |
|---|---|---|---|---|
| Fe | A=0.0036C+0.0007 | 0.9996 | 4.2 | 13.9 |
| Ni | A=0.0093C+0.0009 | 0.9997 | 1.2 | 3.4 |
| Mn | A=0.0062C+0.0003 | 0.9928 | 2.3 | 7.7 |
| Cu | A=0.0447C+0.0022 | 0.9998 | 1.4 | 4.7 |
| Zn | A=0.0556C+0.0017 | 0.9994 | 2.1 | 6.9 |

### Method Validation

Accuracy, precision, sensitivity and limits of detection were determined to assess the validity of the methods used for the digestion and analysis of the honey samples. The precision of the

method was evaluated from the relative standard deviation of the results obtained from repeated measurements made on a given sample for an element while the accuracy was determined by spiking the samples with known concentrations of standard solutions.

The limit of detection of the method was determined from the results obtained from the measurement of three blank samples that were digested and analyzed along with each sample. The limit of detection of the method was calculated as the average blank signal plus three times its standard deviation.

### Extraction of Polyphenols

A 0.5 g honey was extracted with 10 mL of a mixture of methanol to water (70/30, v/v) by maceration at room temperature for 24 h. Then the extracts were filtered through whatman No.42 filter paper. Finally, the filtrate was stored in a refrigerator for further analysis (Doker et al., 2014).

### Preparation of solutions

A Standard stock solution of Gallic acid was prepared by dissolving 0.1 g Gallic acid in 100 mL volumetric flask. Methanol water (v/v) 70:30 (600 mg/L). For the calibration curve, five additional standards of 2.5, 5, 15, 25, 50 mg/L solution were prepared in 25 mL volumetric flask by serial dilution of the stock solution. Then standard solution were stored at 4 ℃ until they were analysis.

For the determination of polyphenol 7.5% $Na_2CO_3$ was prepared by dissolving 7.5 g $Na_2CO_3$ transferring in to 100 mL volumetric flask and adjusting the volume with distilled water up to the mark. Finally it was stored at room temperature.

### Determination of Polyphenol

The polyphenol content in honey was determined using the Folin-Ciocalteu method (Kiros et al., 2016). The reaction mixture was prepared by mixing 0.5 mL of each extract sample was mixed with 0.25 mL of Folin-Ciocalteau reagent, the mixture was allowed to stand for 5 min in dark, 1mL of (7.5 %) $Na_2CO_3$ was added and incubated at room temperature dark place for 90 min. blank solution was prepared parallels containing 0.25 mL Folin-Ciocalteu and 1 mL 7.5% $Na_2CO_3$.The absorbance was measured at 760 nm against blank solution using UV-Visible spectrophotometer. All the measurements were carried out in triplicate for each analysis and a standard curve of gallic acid was prepared for quantification, using a concentration range between 2.5 mg/L and 50 mg/L. Polyphenol content of the honey extracts was expressed in terms of mg GAE/kg.

### Data Analysis

One-way analysis of variance (ANOVA) was used to test the differences among mean values ($p < 0.05$). Data were analyzed by SPSS 20 (IBM Corp, USA).

## RESULTS AND DISCUSSION

### Optimum Conditions for Sample Digestion

Honey samples exhibited the optimum digestion conditions to produce clear solutions suitable for FAAS analysis (Tables 3-5).

**Table 3**. Optimization of reagent volume for the digestion of 0.5 g of honey heated at 150 ℃ for 1:30 h.

| $HNO_3$ (mL) | $HClO_4$ (mL) | Observation |
|---|---|---|
| 3 | 3 | Pale yellow |
| 1 | 5 | Yellow |

| | | |
|---|---|---|
| 5 | 1 | Yellow |
| 4 | 2 | Light yellow |
| 2 | 4 | Clear solution |

**Table 4**. Optimization of time for the digestion of 0.5 g of honey heated at 150 ℃ mixed with 2 mL $HNO_3$ and 4mL $HClO_4$.

| Time (h) | Observation |
|---|---|
| 1:00 | Light yellow |
| 1:20 | Yellow |
| 1:30 | Clear solution |

**Table 5**. Optimization of temperature for the digestion of 0.5 g of honey heated mixed with 2 mL $HNO_3$ and 4mL $HClO_4$ and heated for 1:30 h.

| Temperature ($^oC$) | Observation |
|---|---|
| 100 | Pale yellow |
| 150 | Clear and colorless |

## Analytical Characteristics of the Method

The limit of detection (LOD) of the method ranged from the lowest 1.2 mg/kg for Ni to the highest 4.2 mg/kg for Fe (Table 2).The LOD of the method was below 4.2 −1.2 mg/kg for all the elements and samples studied indicating that the method was useful for the analysis of the elements at trace levels in the honey.

The percentage recoveries were all within the range 83.9–106% across the elements and honey samples (Table 6) indicating that the validity of the digestion method used for the analysis of the elements in the honeys.

**Table 6**. Recovery results (mean ± SD) of metals for spiked samples of honey.

| Metals | Sample concentration (mg/kg) | Amount added (mg/kg) | Amount found after spiking (mg/kg) | (%) Recovery |
|---|---|---|---|---|
| Fe | 54.6 ± 6.0 | 54.6 | 104.3 ± 10.7 | 91.1 |
| Ni | 12.7 ± 0.7 | 12.7 | 26.9 ± 2.4 | 112.5 |
| Mn | 14.9 ± 0.4 | 14.9 | 29.0 ± 0.01 | 94.9 |
| Cu | 12.0 ± 0.7 | 12.0 | 22.1 ± 1.5 | 83.9 |
| Zn | 40.3 ± 2.8 | 40.0 | 83.1 ± 4.8 | 106.9 |

## Concentration of Metals in the Honey Samples

The concentration of five trace metals (Cu, Mn, Ni, Zn and Fe) were determine by using FAAS. The result shows that the metal content of each honey sampling site is different from one to the other. In all honey samples the levels of metals were above the detection limit Table 2.

**Table 7**. Concentration (mg/kg) of the trace metals in honey from the six districts Wollo.

| Sample | Fe | Ni | Mn | Cu | Zn |
|---|---|---|---|---|---|
| Meket | 54.6 ± 6.0 | 12.7 ± 0.7 | 14.9 ± 0.4 | 12.0 ± 0.7 | 40.3 ± 2.8 |
| Wadla | 24.2 ± 2.3 | 8.2 ± 1.0 | 18.3 ± 0.3 | 5.1 ± 0.4 | 36.3 ± 2.0 |
| Gubalafto | 48.7 ± 2.3 | 10.4 ± 0.8 | 21.6 ± 1. 7 | 5.3 ± 0.7 | 35.1 ± 2.1 |
| Delata | 52.7 ± 3.3 | 5.6 ± 0.4 | 15.3 ± 0.7 | 7.4 ± 0.5 | 32.7 ± 3.2 |
| Ambasel | 85.9 ± 2.7 | 10.2 ± 1.0 | 21.8 ± 0.8 | 16.6 ± 0.9 | 53.5 ± 2.8 |
| Tehuledere | 30.2 ± 1.0 | 15.1 ± 0.4 | 25.8 ± 1.5 | 5.6 ± 0.4 | 12.2 ± 0.8 |

## Distribution Pattern of the Trace Metals in Honey Samples

The results showed that the concentrations of trace metals varied among the honey samples collected from different districts. In general, the overall metal levels in the honey samples followed the order: Ambasel > Meket > Delanta > Gubalafto > Tehuledere > Wadla. Considering all samples together, the general abundance pattern of metals was Fe > Zn > Mn > Cu > Ni.

As shown in Table 7, the honey samples from Meket District contained the highest concentration of Fe (54.6 ± 6.0 mg/kg) and the lowest concentration of Cu (12.0 ± 0.7 mg/kg). The decreasing order of metal concentrations in Meket honey was Fe (54.6 ± 6.0 mg/kg) > Zn (40.3 ± 2.8 mg/kg) > Mn (14.9 ± 0.4 mg/kg) > Ni (12.7 ± 0.7 mg/kg) > Cu (12.0 ± 0.7 mg/kg).

In Ambasel District, Fe was also the most abundant metal with a concentration of 85.8 ± 2.7 mg/kg, while Ni had the lowest concentration (10.2 ± 1.0 mg/kg). The increasing order of metal concentrations in Ambasel honey was Ni (10.2 ± 1.0 mg/kg) < Cu (16.6 ± 0.9 mg/kg) < Mn (21.8 ± 0.8 mg/kg) < Zn (53.5 ± 2.8 mg/kg) < Fe (85.8 ± 2.7 mg/kg).

In contrast, honey from Wadla District showed the highest concentration of Zn (36.3 ± 2.0 mg/kg) and the lowest concentration of Cu (5.1 ± 0.4 mg/kg). The decreasing order of metal concentrations in Wadla honey was Zn (36.3 ± 2.0 mg/kg) > Fe (24.2 ± 2.3 mg/kg) > Mn (18.3 ± 0.3 mg/kg) > Ni (8.2 ± 1.0 mg/kg) > Cu (5.1 ± 0.4 mg/kg).

Similarly, in Delanta District, the highest metal concentration was Fe (52.7 ± 3.3 mg/kg) and the lowest was Ni (5.6 ± 0.4 mg/kg). The decreasing order of metal concentrations in Delanta honey was Fe (52.7 ± 3.3 mg/kg) > Zn (32.7 ± 3.2 mg/kg) > Mn (15.3 ± 0.7 mg/kg) > Cu (7.4 ± 0.5 mg/kg) > Ni (5.6 ± 0.4 mg/kg).

For Gubalafto District, Fe (48.7 ± 2.3 mg/kg) was the most abundant metal, while Cu (5.3 ± 0.7 mg/kg) was the least abundant. The decreasing order of metal concentrations in Gubalafto honey was Fe (48.7 ± 2.3 mg/kg) > Zn (35.1 ± 2.2 mg/kg) > Mn (21.6 ± 1.7 mg/kg) > Ni (10.4 ± 0.8 mg/kg) > Cu (5.3 ± 0.7 mg/kg).

In Tehuledere District, Fe (30.2 ± 1.0 mg/kg) had the highest concentration, whereas Cu (5.6 ± 0.4 mg/kg) showed the lowest level. The decreasing order of metal concentrations in Tehuledere honey was Fe (30.2 ± 1.0 mg/kg) > Mn (25.8 ± 1.5 mg/kg) > Ni (10.2 ± 1.0 mg/kg) > Zn (12.2 ± 0.8 mg/kg) > Cu (5.6 ± 0.4 mg/kg).

The observed variation in trace metal concentrations among the honey samples may be attributed to several factors, including geographical location, floral diversity, botanical origin, soil composition, and environmental conditions. In addition, beekeeping practices, honey processing and storage equipment, proximity of bee foraging areas to roads, and the use of agrochemicals such as fertilizers and pesticides may also contribute to differences in metal concentrations in honey samples.

### Comparison of Metal Concentration in Honey Samples with Reported Values

Several studies have reported trace metal concentrations in different types of honey worldwide. Comparing the results obtained in this study with those from other countries provides insight into the quality of Ethiopian multifloral honey in terms of trace metal content and compliance with international guidelines. A summary of this comparison is presented in Table 8.

Iron (Fe) was detected in all honey samples at concentrations ranging from 22.2 to 85.7 mg/kg. The highest Fe concentration was observed in honey from Wadla District, while the lowest was from Ambasel District. The Fe levels in Ethiopian honey were higher than reported values for honey from Slovenia (0.39–70.40 mg/kg) (Golob et al., 2005), Chile (0.1–6.36 mg/kg) (Fredes and Montenegro, 2006), and Croatia (0.50–2.27 mg/kg) (Ursulin-Trstenjak et al., 2015), but lower than those reported for honey from Nigeria (5.00–163.2 mg/kg) (Iwegbue et al., 2015), Morocco (0.88–207.65 mg/kg) (Belouali et al., 2008), Malaysia (55.83–233.6 mg/kg) (Fredes and Montenegro, 2006), South Nigeria (120–342 mg/kg) (Iwegbue et al., 2015), and Egypt (58–3690 mg/kg) (Rashed and Soltan, 2004).

Nickel (Ni) was detected in all samples at concentrations of 5.6–15.1 mg/kg, with the highest in Delanta District and the lowest in Tehuleder District. These values are comparable to reports from South Nigeria (5.00–13 mg/kg) (Omode and Ademukola, 2008). However, they were higher than those reported in honey from Slovenia (0.00–12.7 mg/kg), Nigeria (0.25–6.98 mg/kg), Chile (0.0–1.04 mg/kg), Croatia (0.02–0.11 mg/kg), and Egypt (1.25–4.1 mg/kg).

Manganese (Mn) concentrations ranged from 14.89–25.78 mg/kg, with the highest in Meket District and the lowest in Tehuleder District. The Mn levels were higher than reported for honey from Morocco (0.08–9.76 mg/kg), Chile (0.01–3.14 mg/kg), Croatia (0.09–0.23 mg/kg), and Egypt (0.5–5.7 mg/kg), but lower than those reported for Nigeria (11.00–31.75 mg/kg) and Slovenia (0.12–66.4 mg/kg).

Copper (Cu) was detected at 5.56–15.08 mg/kg, with the highest in Wadla District and the lowest in Ambasel District. The Cu levels were consistent with honey from Slovenia (0.37–15.5 mg/kg) (Golob et al., 2005). Compared to other countries, Cu concentrations were higher than honey from South Nigeria (13.9–21.0 mg/kg), Morocco (0.51–4.75 mg/kg), Chile (0.06–2.0 mg/kg), Croatia (0.07–0.95 mg/kg), Malaysia (ND–2.93 mg/kg), and Egypt (1.0–1.7 mg/kg), but lower than honey from Nigeria (0.25–71.25 mg/kg).

Zinc (Zn) was detected in all samples at 12.2–53.5 mg/kg, with the highest in Tehuleder District and the lowest in Ambasel District. The Zn levels were higher than those reported for honey from Slovenia (0.55–11.2 mg/kg), Morocco (0.04–2.74 mg/kg), Chile (0.01–4.73 mg/kg), Croatia (0.94–30.88 mg/kg), and Egypt (0.5–5.7 mg/kg), but lower than honey from Nigeria (0.25–71.25 mg/kg), Malaysia (4.7–173.77 mg/kg), and South Nigeria (44.6–113 mg/kg).

Overall, the comparison indicates that Ethiopian honey contains appreciable levels of essential trace metals, often higher than those reported in Europe, Chile, and Egypt, but generally lower than some African and Asian honey samples. These findings suggest that Ethiopian honey is

nutritionally valuable and generally within the range reported in international literature, though variations may reflect botanical origin, soil composition, and environmental factors.

**Table 8**. Comparison of the levels of trace metals found in this study with results from other countries.

| Country | Fe | Ni | Mn | Cu | Zn | Reference |
|---|---|---|---|---|---|---|
| Nigeria | 5.00-163 | 0.25-6.98 | 11.0-31.8 | 0.25-71.2 | 1.00-31 | (Iwegbue et al.,2015) |
| Slovenia | 0.30-70.4 | 0.00-12.7 | 0.12-66.4 | 0.37-15.5 | 0.55-11.2 | (Golob et al., 2005) |
| Nigeria | 120-342 | 5.00-13 | NR | 13.9-21.0 | 44.6-113 | (Omode and Ademukola, 2008) |
| Morocco | 0.88-208 | NR | 0.08-9.76 | 0.51-4.75 | 0.04-2.74 | (Belouali et al., 2008) |
| Chile | 0.1-6.36 | 0.01-1.04 | 0.01-3.14 | 0.06-2.00 | 0.01-4.73 | (Fredes and Montenegro, 2006) |
| Malaysia | 55.8-234 | NR | NR | ND-2.93 | 4.7-174 | (Moniruzzaman et al., 2014) |
| Croatian | 0.50-2.27 | 0.02-0.11 | 0.09-0.23 | 0.07-0.95 | 0.94-30.9 | (Ursulin-Trstenjak et al., 2015) |
| Egypt | 5.7-3690 | 1.25-4.1 | 0.5-5.7 | 1.00-1.70 | 5.00-9.3 | Rashed and Soltan et al., 2004) |
| North and South Wollo, Ethiopia | 24.2-85.8 | 5.6-15.1 | 14.9-25.8 | 5.1-16.6 | 12.2-53.5 | This study |

## Polyphenol Contents

In this study, the polyphenol content was determined spectrophotometric according the Folin-Ciocaltu method (Blainski et al., 2013).

The highest concentration of total polyphenol was found in Meket district with the value of 1221.7± 23.0 mgGAE/kg and lowest concentration of total polyphenol was found in Tehuledere with the value of 519.8 ± 6.1 mgGAE/kg. The concentration polyphenol in Meket almost twice of the polyphenol of in Tehuledere. The levels of total polyphenol was decreasing order of the average concentration in sampling area as follows Meket > Ambasel > Gubalafto > Delanta > Wadla > Tehuleder. The increasing order total polyphenol content in North Wollo zone was Wadla < Gubalafto < Meket district and the increasing order total polyphenol content in South Wollo zone was Tehuledere < Delanta < Ambasel district. Total polyphenol content in mgGAE/kg North Wollo was 893.5 and the South Wollo was 707.3. Therefore North Wollo was highest total polyphenol than South Wollo zone. The highest total polyphenol content in the investigated North Wollo zone honey confirmed the good quality of the honey. The variation in the phenolic content for our

samples compared to those from different countries may be due to the different geographical and botanical sources of honey.

**Table 9**. Polyphenol concentration (mean ± SD mgGAE/kg) measured in the honey samples from different districts of North and South Wollo zones.

| Sample | Concentration |
|---|---|
| Wadal | 628.6 ± 6.6 |
| Meket | 1221.7± 23.0 |
| Gubalafto | 830.1 ± 32.2 |
| Tehuledere | 519.8 ± 6.1 |
| Delanta | 756.5 ± 27.6 |
| Ambasel | 845.4 ± 26.4 |

## Comparison of the Polyphenol Levels with Literature Value

The polyphenol in this study ranged between 519.8-1221.7 mg/kg f. The total polyphenol content the lowest 519 mg/kg in Tehuledere and the highest 1221.7 mg/kg total polyphenol content in Meket. Generally our samples show higher phenolic content compared to honey from other countries; reported Brazil 250-548 mg/kg ( Joniorison et al., 2014), Slovenia honey 44.8-241 mg/kg (Bertoncelj et al., 2007), Czech honey 39.2-167.1 mg/kg (Lachman et al., 2010), Malaysian honey 305.5-419.9 mg/kg (Khalil et al., 2012) and lower polyphenol content compared to honey from reported for Mexico honey 621.3-368.4 mg/kg (Bedassa et al., 2017). The most similar phenolic contents to was reported for Moroccan 239.5 -1138.5 mg/kg (Benlyas et al., 2016) and Argentina honey 401.8-1188.2 mg/kg (Cabrera et al., 2017).

**Table 10**. Comparison of the concentration of polyphenol in honey found in this study with results from other countries.

| Country | Polyphenol (mgGAE/kg) | Reference |
|---|---|---|
| Mexico honey | 621.3-368.4 | (Bedassa et al., 2017) |
| Moroccan honey | 239.5-1138.5 | (Benlyas et al., 2016) |
| Brazil honey | 250-548 | ( Joniorison et al 2014) |
| Slovenia honey | 44.8-241.4 | (Bertoncelj et al., 2007) |
| Argentinian honey | 401.8-1188.2 | (Cabrera et al., 2017) |
| Czech honey | 39.2-167.1 | (Lachman et al., 2010) |
| Malaysian honey | 305.5-419 | (Khalil et al., 2012) |
| North and South Wollo Ethiopian honey | 519.8-1221.7 | This study |

## CONCLUSION

This study determined the concentrations of five trace metals (Fe, Ni, Mn, Cu, and Zn) and the total polyphenol content in honey samples collected from selected districts of North and South Wollo Zones, Ethiopia. The concentrations of the analyzed metals (mg/kg) ranged from 24.2–85.9 for Fe, 5.6–15.1 for Ni, 14.9–25.8 for Mn, 5.1–16.6 for Cu, and 12.2–40.3 for Zn. Among the analyzed metals, Fe showed the highest concentration in most districts except Wadla, while Cu exhibited the lowest concentration in most samples except those from Ambasel district. The analytical method demonstrated good accuracy, with percentage recoveries ranging from 83.91% to 112%, indicating acceptable performance of the digestion and analytical procedures. Statistical analysis using one-way ANOVA showed that there was a significant difference ($p < 0.05$) in the concentrations of the trace metals among the honey samples collected from the different sampling sites. The total polyphenol content of the honey samples ranged from 519.8 to 1221.7 mg GAE/kg. The mean total polyphenol content was 893.5 mg GAE/kg in North Wollo and 707.3 mg GAE/kg in South Wollo. Among the studied districts, the highest polyphenol content was observed in Meket, while the lowest was recorded in Tehuledere. Overall, the results indicate that honey produced in the study areas contains appreciable amounts of essential trace metals and polyphenolic compounds, which contribute to its nutritional value and antioxidant potential. The findings also provide baseline information on the mineral composition and bioactive properties of honey from North and South Wollo Zones, which may be useful for future studies on honey quality assessment and environmental monitoring.

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