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Determination of selected essential and non- essential metals of honey in wolaita zone, southern Ethiopia

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Abstract

Honey is a sweet natural product produced by bees. It has many beneficial health promoting effects. The essential and non-essential metals' contents of bee honey samples collected from Damot Gale district were determined by atomic absorption spectrophotometer. The samples were digested to obtain the essential and non-essential metals by optimum digestion condition. The digestion method for honey analysis was found to be efficient for all in the analyzed metals. The analysis was validated through the recovery experiments showing that a good percentage recovery (95.7-103%). The results obtained from honey samples collected from the bee farmers revealed that the values of pH, moisture and ash content of mid land to be 3.65 ± 0.062, 16.77 ± 0.06 and 0.42 ± 0.006, respectively while the low land part is 3.88 ± 0.057, 16.97 ± 0.034 and 0.46 ± 0.003, respectively. The results of this study revealed that the concentrations of most metals are higher in the honey samples collected from low land part than mid land part within the selected sites. This study's result was compared with other reported values and found almost comparable. The results of current finding were also compared with international standards set by WHO/FAO and revealed that the levels of some essential and non- essential metals in honey samples were within permissible limits. Therefore, the finding of this study indicated that the honey of the study areas was found safe for consumption regarding studied metal concentration.

Keywords: Essential and non- essential metals, Proximate analysis, Honey and Atomic Absorption Spectroscopy (AAS)

Introduction

Honey is the natural sweet substance produced by honey bees from the nectar of blossoms, from secretion of living parts of plants (Abebe et al., 2016). The minor components in honey include amino acids, vitamins, organic acids and minerals (Shobham et al., 2017). The difference on botanical origin of honey is one of the matters that can characterize differences among color,

taste, flavor and content of physiologically active ingredients (Silva et al., 2009; Bogdanov et al., 2008).

Essential metals in honey such as Ca, Cu, Zn and Ni play a vital role in normal life activities like in lipid metabolism, blood building, hormone functioning, wound healing, proper hair growth, etc. Also, nutritionally, honey has a great variety of essential elements. Some of them are present in trace levels, and could be toxic if they exceed safety levels (Solayman et al., 2016). Heavy metals such as Pb, Cd, and Hg are classified as trace element and toxic nature even in minute quantities. Any non-essential or heavy metals present in honey above the admitted levels by pollution standards are threats to human body through the possible negative effect of the contaminants (Spiteri et al., 2016).

In a given agro ecology, the relative heavy metal's concentration depends on the element, plant species, soil type, site characteristics, management practices, and geology (Adriano, 1986). There are two main routes by which trace elements enter into agro ecology. These are aerial (e.g., aerosols, particulate matter, resuspended and airborne dusts, etc.) and land (fertilizers, pesticides, solid waste, wastewaters, other soil amendments, etc.) (Adriano, 2001) These may have differences in heavy metal concentrations of honey bee from different agro ecologies.

Many reports indicated that honey samples collected from industrial regions had the higher heavy metal (Cd, Pb, Hg, Zn, Cu, Ni and Cr) concentrations than those from natural or non-industrial regions because of high heavy metal wastage in it. The accumulation of the toxic metal in human body causing the side effects so honey quality specific elemental content was the important factor for human (Khan et al., 2015; Mohmoud et al., 2015). The bioavailability of the essential elements depends on their chemical forms, the composition of diet and health situation of individuals (Frieden, 1985). Thus, establishment of the optimum daily requirements and determination of actually daily intake of essential elements are important problems. In absolute deficiency of essential elements, death may result with limited intake (Solayman et al., 2016).

Honey is the results of a bioaccumulation process useful for the collection of information related to the environment where bees live. Since the forage area of the hive is more than 7 Km^2 and the bees come in contact with air, soil and water, the concentration of metals in honey reflects their amount in the whole region. Therefore, honey has been recognized as a biological indicator of environmental pollution (Kostic et al., 2015). The composition of honey depends on the quality of honey and its specific floral and vegetation in the area from which the honey originates and

the diversity of the ecosystem in which the bees are kept, specifically in non-industrial areas and the components of honey are important for judging honey quality (Haftu, 2015).

Ethiopia is one of the homes for farming practices of honey. Thus, the country produces honey from various an ecological and climatic conditions. Therefore, honey is used as an additional source of income for farmers, next to cropping and livestock rearing (Ashenafi, 2018). The annual honey production of Ethiopia is estimated to be 45,300 metric tons. It ranks the country first among honey producing Africa's countries and tenth in the rest of the world (CSA, 2012; FAO, 2010). According to literature's reports, inadequate of production knowledge and poor post-harvest handling system often result poor honey's quality (Taye and Verschuur, 2014).

As mentioned above, honey possesses numerous essential and non-essential elements which contain nutritional, healing and preventive properties. These are a direct consequence of its chemical composition. In order to have a beneficial effect, honey must be free from any contaminating agents such as toxic and heavy metals above the permissible limits that results in various health problems for the consumers. However, in the study areas, people prefer mid land sites' honey rather than the low land sites' honey. As a result, mid land sites' honey is used widely. However, both the preference and usage was not supported by research. Thus, it is possible to say that the practices were made without any justified reason. So that, the comparative study on the determination of selected essential and non- essential metals of honey in the study area is very vital. Therefore, the objective of the study is to determine selected essential and non- essential metals of honey in the study area in different ecology.

Materials and methods

Sample site description, collection and transportation of sample

The study was conducted in Wolaita Zone, Damot Gale district. Wolaita Zone is located in the Southern Nation Nationalities and Peoples Region (SNNPR). It is 390 km away from Addis Ababa, the capital city of Ethiopia. The total population of the zone is estimated to be 1, 528,000. It is one of the most densely populated zones. Wolaita Zone has 15 administrative districts. Damot Gale, one of its administrative districts is located 165 km south-west of Hawassa town. According to the Statistical Agency of the Damot Gale finance and economic development office, the district is situated in 6^0 88'-7^0 11' north latitude and 37^0 74'-38^0 00' east longitude. The district is adjacent to Misrak Badawacho (Hadiya zone) and Damot Pulasa district in the north,

Boloso Sore and Damot Sore district in the west, Sodo zuria district in south, Duguna Fango and Damot woyde district to the east. The district covers the area of 242.85 square kilometers or 24,127.3 hectares. Ecologically, 25.8, 61.2 and 13 % of zone is high land ('dega'), mid land ('woynadega'), and low land ('kola'), respectively and the population settlements of the district per kilometers square is 694.9. The major farming system in the study area is small holder mixed farming system. The bee honey production is common in both mid land ('woynadega'), and low land ('kola') of the study area (CSA, 2012).

The honey samples were collected from selected six kebeles of Damot Gale District (Shasha Gale, Wogera, Bala Koysha, Harito Kontola, Harito Burkito and Taba) in Wolaita Zone. The selection was made based on the criteria of relatively high number of honey production capacity information from livestock and fishery department of the Damot Gale district office. From each kebele, five farmers were selected for honey sampling. Therefore, a total of 5 farmers were selected randomly for collection of honey samples from each kebele. From a single farmer, 200 gm of honey sample was collected in polyethylene container. As a result, from each kebele, 1000 gm honey was collected as 30 samples. The honey was placed in tightly closed plastic containers and stored in cool place.

From each site, 100 ml of honey samples were collected in to 500 ml pre-washed (soaked in 20% in HNO_3 and rinsed with deionized water) polyethylene bottle to avoid potential contamination and the sample were kept in an ice box. Liquid honey samples were mixed thoroughly by stirring or shaking to remove foreign matters such as wax, sticks, bees, particles of comb etc. The samples were heated to 40^0C in water bath and strain through cheese cloth in hot water funnel to get a sample representative of each kebele (Beyene and Marco, 2014). The sampling criteria are shown below (Table 1).

Table 1: The sampling criteria used in the study

Agro ecology	Sample site	Number of farmers
Mid land	Shasha Gale	5
	Wogara	5
	Bala Koysha	5
Low land	Harto Burkito	5
	Harto Kontola	5
	Taba	5

The samples were sealed in polyethylene container, labeled properly and were made ready for analyses. These six samples were preserved in polyethylene container at 4-5^{0}C in the refrigerator.

Sample preparation

The preserved honey samples were solidified in deepfreeze. From the solid sample 500 mg were directly weighed and optimization of digestion procedure carried out in this sample.

Optimization of digestion procedure

To prepare a clear colorless sample solution, suitable for the analysis using AAS, different working procedures for the digestion of honey samples were optimized. The optimization was made using nitric acid (HNO_3) and perchloric acid ($HClO_4$) mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature (Huang et al., 2004). Perchloric acid digestion can extract metals from honey samples but it is a more complicated procedure and requires special facilities and safety precautions. The optimum digestion procedure was selected depending upon, the clarity of digests (solution without any residue and suspended matter), and minimal reagent, volume consumption, minimal digestion time, power and temperature (Chen and Ma, 2001).

The digestion procedures for honey samples were given in Table 2. From the twelve steps in Table 2, those which giving clear solution with minimal temperature, time and volume were selected and used to optimize digestion procedures by keeping other parameters. The optimum digestion condition for honey sample was indicated under number 4, 8 and 12 and used throughout the whole analyses (Endalamaw and Chandravanshi, 2015).

Digestion of samples

A mass of 500 mg of each honey sample was transferred to digestion vessels followed by addition of 3 ml of concentrated HNO_3 and 3 ml of $HClO_4$. The vessels were carefully shaken and placed in a fume hood for about 20 min for pre-digestion. Then, the pre-digested samples in the digestion vessels were closed and heated following the optimized procedure. The addition of 3 ml of concentrated HNO_3 and 3 ml of $HClO_4$ applied and the temperature was kept at 270 ^{0}C for 180 min by rotating the power to 80 W. Then, to avoid splashing or spraying which cause sample loss, the digestion vessel was cooled at room temperature (for about 20 min Finally, by

opening the digestion vessel carefully the digested solutions were transferred to 25 ml volumetric flask and made up of the mark by deionized water. The digested samples were then kept in a refrigerator until analyzed by Atomic Absorption Spectroscopy (AAS).

Table 2: The optimization of digestion procedure using volume, temperature and time in honey samples

Reagents	Volume of Reagents (ml)	Temperature (^{O}C)	Time (min)	Observation
HNO_3	6	270	180	Deep Yellow solution
HNO_3: $HClO_4$	5:1	270	180	Light Yellow solution
HNO_3: $HClO_4$	4:2	270	180	Yellow solution
HNO_3: $HClO_4$	3:3	270	180	Colorless solution
HNO_3: $HClO_4$	3:3	180	180	Yellow solution
HNO_3: $HClO_4$	3:3	210	180	light yellow solution
HNO_3: $HClO_4$	3:3	240	180	light yellow solut ion
HNO_3: $HClO_4$	3:3	270	180	Colorless solution
HNO_3: $HClO_4$	3:3	270	90	Yellow solution
HNO_3: $HClO_4$	3:3	270	120	Light Yellow solut ion
HNO_3: $HClO_4$	3:3	270	150	Yellow solution
HNO_3: $HClO_4$	3:3	270	180	Colorless solution

Instrument operating conditions

In this study, six elements were selected to study the concentration of metals in honey using atomic absorption Spectrophotometer equipped with deuterium arc back ground corrector and air-acetylene flame system using external calibration curve. For each metals their respective hallow cathode lamp was inserted in to the atomic absorption spectrometer and the solution was successively aspirated in to the flame. Three triplicate determinations were carried out for each sample. Six elements (Ca, Cd, Cu, Pb, Zn and Ni) were analyzed by absorption mode of the instrument. The same analytical procedure was employed for the determination of elements in the six digested blank solutions. Calibration of the instrument was repeated periodically during operation. The adjustment of instrument parameters is very important for getting maximum

signal intensity with minimum noise. The operating conditions for AAS that were employed for each metal are given below (Table 3).

Table 3: Instrument operating conditions

Metals	Wave length (nm)	Slit width (nm)	Lamp current (mA)	IDL (ppm)	PMT (v)
Ca	422.7	1.2	3	0.005	300
Cd	228.8	1.2	2	0.0024	269.3
Cu	324.8	1.2	2	0.007	259
Pb	283.3	1.2	2	0.06	298
Ni	232	0.2	3	0.014	279
Zn	213.9	0.5	2	0.0024	386

Instrument calibration

The qualities of results obtained for major essential and non-essential metal analysis using AAS are seriously affected by the calibration and standard solution preparation procedures. The instrument was calibrated using four serious of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediated solutions. Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed below (Table 4). The correlation coefficients for all the calibration graphs were above 0.999 which shows that there is linear relationship between the concentration and absorbance of metals.

Method Detection Limit

Method detection limit (MDL) is defined as the minimum concentration of analyte that can be measured and reported with 95 % confidence that the analyte concentration is greater than zero, but it may not necessarily be quantified as an exact value (Harris, 1982). The method detection limits was calculated by multiplying the standard deviation of the blank readings (n=6) by three and presented in Table 5.

MDL=3×SB; Where SB is standard deviation of the blank

Table 4: Concentrations of working standard solutions and correlation coefficients of the calibration curves

Metal	Concentration of intermediate standard solution (mg/L)	Concentration of working standard (mg/L)	Correlation coefficient (R^2)	Equation of calibration curve
Ca	10	0.25, 0.5, 1, 2	0.9999	Y =0.0644x + 0.005
Cd	10	0.25, 0.5, 0.75, 1	0.9994	Y= 0.3953x + 0.0017
Cu	10	0.25, 0.5, 1, 2	0.9999	Y = 0.386x + 0.0025
Pb	10	1, 2, 3, 4	0.9998	Y= 0.0303x + 0.0022
Ni	10	1, 2, 3, 4	0.9988	Y= 0.1476x + 0.0093
Zn	10	0.25, 0.5, 0.75, 1	0.9998	Y = 0.6762x + 0.006

Table 5: The method detection limit for honey samples (n=3 for all metals)

Metals	MDL (mg/L)	IDL (mg/L)
Ca	0.00508	0.005
Cd	0.00334	0.0024
Cu	0.00943	0.007
Pb	0.069	0.06
Ni	0.0144	0.014
Zn	0.0112	0.0024

MDL - Method detection limit IDL - instrument detection limit

Method validation

Since there is no certified reference material (CRM) in the laboratory, the validity of the optimized digestion procedure was checked by spiking experiment on Damot Gale district honey sample. As shown in Table 5, the results of the recoveries for the metals in the honey samples lie within the range of 95.7 - 103 %. This indicates that recovery results were within the acceptable range (80-120 %). Consequently, the proposed method for digestion of honey samples was valid and reliable.

Recovery test

As shown in Table 6, the results of percentage recoveries for the studied metal in honey were within the acceptable range (95.7-103.0%) in the honey samples. Method validation is the process of providing that analytical method is acceptable for its intended purpose. The validity of the optimized digestion procedure for honey was checked by carrying out spiking and the analysis calculated by using the formula given below and tabulated.

$$\% \text{ Recovery test} = \frac{\text{concentration of spiked} - \text{concentration of unspiked}}{\text{amount added}} \text{x} 100$$

Table 6: Recovery test for the optimized procedure of honey sample

Metals	[a]Concentration in sample (mg/L)	Amount added (mg/L)	[b]Concentration in spiked (mg/L)	[c]Recovery %
Ca	46.01 ± 0.09	0.50	46.49 ± 0.013	95.7 ± 2.56
Cd	0.16 ± 0.005	0.50	0.663 ± 0.003	99.5 ± 0.42
Cu	1.74 ± 0.012	1.00	2.74 ± 0.022	99.3 ± 2.27
Ni	2.83 ± 0.034	1.00	3.866 ± 0.033	103 ± 3.25
Zn	4.79 ± 0.03	0.50	5.29 ± 0.019	98.4 ± 3.81

[a] Mean concentration ± SD of samples analyzed in triplicate.

[b] Mean concentration ± SD of samples spiked in triplicate.

[c] Mean recovery ± SD of percentage recoveries of triplicate analysis.

Determination of ash content

Honey samples were ashed by calculation in a furnace at 500- 600 ^{0}C to a constant weight. Ash percentage was calculated for all honey samples. The percentage of ash content (PAC) was determined by heating 5 gm of the honey sample in the muffle furnace at a temperature of 550^{O}C for 3 h and weighed after cooling in desiccators for 30 minutes to obtain the weight of ash.

$$\% \text{ Ash} = \frac{(\text{Wt. of crucible} + \text{ash}) - \text{Wt. of empty crucible}}{\text{wt. of honey sample}} \times 100$$

Determination of moisture content

Each honey samples were weighed accurately in a pre-weighed crucible dish and gently heated in oven at 105^{0}C until the sample turned black and dried. This was allowed to cool in desiccators and re-weighed again until a constant weight will be obtained. The weight loss in respect of 100

g represented the moisture contents of the honey sample. The percentage moisture content (MC) was calculated for all samples using the formula below.

% MC = $\frac{M1-M2}{M1-Mo}X100$ where Mo = the weight of dish, M_1 = wt. (g) of dish of honey sample before drying and M2 = wt. (g) of dish of honey after drying

Determination of pH

The pH of honey samples were determined by measuring out 10 ml of each honey sample into a clean beaker and adding 2 -4 ml of H_2O_2, its pH was determined using a pH meter.

Measuring mineral elements from honey

The honey samples were analyzed for mineral elements such as Cu, Ca, pb, Cd, Ni and Zn using AAS. Atomic absorption spectrophotometry was used in order to determine the concentration of metals at different wavelengths. Standards were prepared for each of the elements.

Data analysis

Data analyses were performed using the statistical analysis system. The bee honey data generated were subjected to analysis of variance (ANOVA) using the general linear model procedure. Least significant (LSD) test was used to determine the differences among honey samples from different sites based on the metals level at P = 0.05. The simple correlation analysis of data was computed to determine the relation of each metal level with other metals level in the honey.

Results and Discussion

Ash content

As indicated in Table 6, the ash contents of honey in the study area ranged between 0.37± 0.012 and 0.47± 0.004%. The maximum allowable ash contents of honey according to the specifications of Ethiopian, European and Codex Alimnetarius standards are <0.6, <0.6 and < 0.8 %, respectively. Therefore, the ash contents in all analyzed honey samples were below the limit (0.6 %) allowed by different standards and Ethiopian Standard. The highest value of ash content was recorded for Harto Burkito site honey samples (0.47 %) while the lowest value recorded for Shasha Gale site was (0.37%). The mean separation method showed that the variation of the average content between different sites was statistically significant (P<0.5).This may be due to

different agro ecological factor (Alemayehu et al., 2016). However, the ash content of all in sites below critical level indicates that good honey quality.

Moisture content

Water content is a parameter that is related to the climatic conditions, the season of the year and degree of maturity (Mohamed et al., 2013). The present study indicates that the moisture contents vary between 16.43 % (Bala Koysha) and 17.34 % (Shasha Gale) (Table 6) and the others were also found to be between these ranges. Mean separation method indicates that the moisture content in the honey samples differ significantly among sites that honey samples collected ($P< 0.05$) (Table 7). This difference may be attributed to the differences in botanical origins, weather conditions and original moisture contents (Alemayehu et al., 2016; Mohamed et al., 2013).

The pH of the honey samples

The mean pH values recorded were: 3.53, 3.66, 3.75, 3.91, 3.85 and 3.88 for Shasha Gale, Wogara, Bala Koysha, Harto Kontola, Harto Burkito and Taba honey samples, respectively (Table 7). The result of this study confirmed that the pH values of all honey samples were found to be acidic (<7) and line with the finding of Yohannes et al. (2018). There was statistical significant difference ($P< 0.05$) among the pH of honey samples analyzed. The pH values of the samples were also within the accepted range (3.5 –5.5) according to the same standard (Salah and Samah, 2015). The pH of the honey samples in the study area were found to be comparable with previous findings such as the pH of honey samples collected from Saudi Arabia (3.88–4.25) (Osman et al., 2007), Czech Republic (3.70–4.40) (Čelechovská and Vorlova, 2001), Chile (3.79–5.08) (Fredes and Montenegro, 2006), Croatia (4.21-5.55) (Daniela et al., 2008), Argentina (3.19–4.06) (Naab et al., 2008) and Ethiopia (Yohannes et al., 2018).

Distribution of metals in honey samples

In the present study, the concentration of essential and non-essential (heavy) metals (Ca, Zn, Ni, Cd, Cu and Pb) in honey samples were determined using AAS. Among the analyzed metals, Ca, Zn, Cu, Ni and Cd were detected in all samples whereas lead (Pb) was not detected. This may be due to its concentration which is below the method detection limit. This indicates that the honey of the study area is good in terms of Pb concentration since it has no beneficial role in human

metabolism and produces a progressive toxicity and can cause health disorders (Chandrama et al., 2014). As it is shown in Table 8, the concentration of calcium ranged from 44.95 mg/L to 47.11 mg/L, the lowest concentration (44.95mg/L) was found in honey sample collected from Shasha Gale site while the highest concentration (47.11mg/L) from Harto Burkito. The content of Ca, found in this study, was found to be comparable with permissible levels set by FAO/WHO (40-100 mg/L) in honey (FAO/WHO, 1999).

Table 7: The moisture, ash and pH of honey sample of Damot Gale district

Parameter	Mean ± SD across sampling sites							
Sampling sites	Shasha Gale	Wogara	Bala Koysha	Harto Kontola	Harto Burkito	Total	CV	LSD
Moisture content (%)	17.34^{e}± 0.097	16.53^{d}± 0.031	16.43^{d}± 0.051	17.07^{b}± 0.06	16.95^{c}± 0.006	16.90^{c}± 0.035	0.323	0.097
Ash content (%)	0.43^{c}± 0.0034	0.37^{d}± 0.012	0.45^{b}± 0.0035	0.46^{ab}± 0.003	0.47^{a}± 0.004	0.45^{b}± 0.0029	1.84	0.015
pH	3.53^{c}± 0.15	3.66^{b}± 0.016	3.75^{b}± 0.02	3.91^{a}± 0.01	3.85^{a}± 0.153	3.88^{a}± 0.007	1.76	0.012

The mean separation method by least significant difference (LSD) indicates that the distribution of calcium metal in honey samples showed statistically significant difference among sites. This may be due to differences in the agro ecology where honey samples were collected. Moreover, as compared with the metals in the samples of honey, there was higher content of calcium determined. The result of this study lines with the finding of Solayman et al (2016), who reported that most abundant major elements in honey samples are potassium and calcium.

The concentration of cadmium ranged from 0.15 to 0.17 mg/L and it was the least abundant element in the honey samples studied. The higher Cd (0.16mg/L) content was obtained in honey samples from Bala koysha and wogara while the lower Cd (0.15mg/L) concentration was obtained in honey samples from Taba areas. Significance difference was found in Cd content among Wogara, Bala Koysha and Taba honey. However, the other groups Shasha Gale, Harto Kontola and Harto Burkito honey Cd content did not show statistically significant difference among each other. Relatively high CV (2.56) may be due to variation in botanical origin, honey

nectar, climatic variables such as temperature. In all honey samples, Cd content is in permissible level because it is below critical level (0.2 mg/L) (Kaspchak, 2015).

The concentration of copper ranged from 1.69 to 1.77mg/L (Table 8). The lowest concentration of copper (Cu) (1.69mg/L) was found in honey samples collected from Bala Koysha areas while the highest concentration of Cu (1.77mg/L) in Harto Kontola honey. The Cu concentration of Shasha Gale and Taba honey are not significantly different. In addition, Harto Burkito and Harto Kontola honey Cu concentration do not show significant difference. However, in Bala Koysha and Wogara honey samples, Cu concentrations were significantly different. The content of Cu reported in this study was generally found to be permissible level because it is less than the critical level of Cu in food set by WHO/FAO (2 mg/L) in honey (FAO/WHO, 1999). The deficiency of copper leads to low white blood cells and poor growth while excess intake of copper can cause vomiting, nervous system disorder and Wilson's disease (Buba et al., 2013).

The level of nickel ranged from 2.44 mg/L to 3.21 mg/L where the minimum (2.44 mg/L) concentration was observed in Shasha Gale site and the maximum concentration (3.21 mg/L) was obtained from honey samples of Taba site. The nickel concentrations of Shasha Gale (2.44±0.0013) honey sampling sites are significantly different from all the others. Wogara (2.57±0.0014) and Bala Koysha (2.49±0.0008) honey Ni concentration was not significantly different and also Wogara honey Ni concentration is higher than that of Bala Koysha. Similarly, Harto Kontola (3.13±0.0025), Harto Burkito (3.18±0.0029mg/L) and Taba (3.21±0.0016 mg/L) (Table 8) honey Ni concentration do not show significant difference. The Ni concentration in Harto Kontola honey (3.13±0.0025 mg/L) was significantly lower than Harto Burkito (3.18±.0.0029 mg/L) and also Ni concentration in Harto Burkito honey was significantly lower than Taba (3.21±0.0016 mg/L).

The concentration of Zinc was found to be in the range of 4.49 mg/L to 5.13 mg/L and the lower and the higher concentration of it was found in Bala Koysha and Harto Kontola sites, respectively. The Harto Kontola (5.13±0.0064) honey zinc (Zn) concentration was significantly higher than from all the sampled sites Sh.Gale (4.50±0.0019), Taba (5.03±0.0012), Wogara (4.5±0.0008), Bala Koysha (4.49±0.0019) and Harto Burkito (5.12±0.0016). The honey zinc concentration of Shasha Gale (4.50.96±0.0019), Bala Koysha (4.49±0.0019) and Wogara (4.5±0.0008) significantly lower than Taba (5.03±0.0012), Harto Burkito (5.12±0.0016) and Harto Kontola (5.13±0.0064). However, Harto Burkito, Taba, Shasha Gale, Wogara and Bala

Koysha the honey zinc concentration was significantly higher accordingly, in the descending order one another. The Zn concentrations in honey samples collected from Ha.kontola and Ha. Burkito did not show statistically significant difference (Table 8). Similarly, Zn concentrations do not have significant difference among each other in Shasha Gale, Wogara and Bala Koysha honey. Finally, lead (Pb) was not detected because it was below detection limit in all six sampling sites of honey.

Table 8: Mean concentration (mean ± SD) of essential and non-essential metals in honey samples of Damot Gale district

Metals	Mid land			Low land			CV	LSD	F-value	P-value
	Shasha Gale	Wogara	Bala Koysha	Harto Kontola	Harto Burkito	Taba				
Ca	44.95^{f}± 0.0052	45.01^{e}± 0.005	45.11^{d}± 0.0013	46.97^{b}± 0.0108	47.11^{a}± 0.0029	46.89^{b}± 0.0012	0.014	0.011	89,953.6	0.05
Cd	0.16^{ba}± 0.0018	0.17^{a}± 0.0002	0.17^{c}± 0.0001	0.16^{ba}± 0.0009	0.16^{ba}± 0.0015	0.15^{b}± 0.0018	2.56	0.0074	2.3	0.05
Cu	1.71^{b}± 0.39	1.70^{c}± 0.006	1.69^{d}± 0.0026	1.77^{a}± 0.0016	1.76^{a}± 0.0035	1.75^{b}± 0.0014	0.385	0.012	78.15	0.05
Pb	ND	ND	ND	ND	ND	ND	-	-	-	-
Ni	2.44^{c}± 0.0013	2.57^{b}± 0.0014	2.49^{b}± 0.0008	3.13^{a}± 0.0025	3.18^{a}± 0.0029	3.21^{a}± 0.0016	2.49	0.13	81.55	0.05
Zn	4.50^{c}± 0.0019	4.50^{c}± 0.0008	4.49^{c}± 0.0019	5.13^{a}± 0.00064	5.12^{a}± 0.0016	5.03^{b}± 0.0012	0.086	0.0073	18,246.8	0.05

*Means with the same letters are not significantly different. *LSD: least significant difference

* Results are presented as Mean ± Standard deviation *CV: coefficient of variation

*ND: not detected

Pearson correlation of metals within honey sample

To determine the relationship between the essential and non-essential metals concentration in the honey samples, Pearson product moment correlation analysis was conducted and presented in Table 9. There was statistically significant a negative correlation between cadmium and all the other metals concentration of honey. This significant and negative correlation may indicate the

uptake of Cd inhibited by other metals. However, copper showsed a significantly strong positive correlations as copper and calcium, r = 0.957; copper and zinc, r = 0.978; copper and nickel, r = 0.936 at $p < 0.05$ level. Similarly, calcium showed positive and strong significant relation with Zn and Ni with r value 0.996 and 0.998, respectively.

Table 9: The relationship between essential and non-essential metals of sampled sites

	Cadmium	Copper	Calcium	Zinc	Nickel
Cadmium	1	-0.702	-0.683	-0.678	-0.705
Copper	-	1	0.957**	0.978**	0.936**
Calcium			1	0.996**	0.988**
Zinc				1	0.981**
Nickel					1

Conclusion

The optimized digestion condition for analysis of essential and non-essential metals from honey samples was found to be effective for all metals. In addition, the optimum condition was validated through spiking experiment from which good percentage recovery (95.7-103 %) that was obtained for the identified metals. The results of this study indicated that calcium had highest concentration.

When comparing the result of present study with other literature reported values, almost they are comparable. There is no toxicity of heavy metals observed in the honey samples of the study area. As observed from the results of this study, the concentrations of most metals are higher in the low land part honey samples than mid land part of honey. Furthermore, the results obtained in the present work are almost similar with those reported in other countries. On the other hand, metal Pb was not detected in none of honey samples collected from low land or mid land sites. Therefore, the result (i.e Ca, Ni, Zn, Cd and Cu) obtained in this study showed that the honey produced in selected sites of Damot Gale Distrrict were suitable for health and can be used baseline data for further investigations.

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