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Study on Pesticide Residues and Heavy Metals Levels in Honey Samples Collected from Walmara District of Oromia Special Zone, Ethiopia - ə

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ABSTRACT

Many information have shown about the nutritional and medicinal characteristics of honey but it may contain compounds that may lead to toxicity due to contamination of compound not naturally found in honey that has been added to honey t from chemicals discharged from agricultural activities or factories. The objective of this study was to investigate pesticide residues and heavy metals levels in honey from Walmara district of Oromia Special Zone, Ethiopia. The analysis of Zn, Cu, Pb, Cd, Ni and Cr were obtained by using flame atomic absorption spectrophotometer indicated that the concentrations ranged from 1.41-6.94 µg/ g, 0.22-1.22 µg/ g, 0.37-0.90 µg/ g, 0.04-0.70 µg/ g, 0.26-0.60 µg/ g and 0.16-0.50 µg /g with mean concentration ranges, respectively. However, Fe was determined by using UV-visible Spectrophotometer and the value range was 4.87-11.79 µg/ g. The percentage recovery for metal analyses was from 85% to 104%. Concentrations of Cd, Cu, Cr and Pb in honey samples from various study sites were not significantly different but Fe, Zn and Ni levels were significantly different at (p = 0.05). Pesticide residues analyzed in the AS, BWB, GSL and WC honey samples were aldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, P,P'DDD, P,P'DDE, P,P'DDT, O, P-DDD, endosulfan, endrine, heptachlor, heptachlor exo-epoxide and diaznone were determined by using gas chromatography coupled with mass spectrometer technique. The residues in all samples were found to be below detection limits. The detection limits range was from 0.001 to 0.017 ng/g for the residues analyzed while the recovery percentages were range from 75-105%. Most trace metals and all residues have been found to be within the acceptable range set by National and International Standards except Pb and Cd contents in some samples which may be due to availability of cement factory near the sampling site. Thus focusing on the way of controlling the pollutants is important in the study area. In addition, further checking studies must be made to improve honey safety and protect users' healthiness.

Keywords: Flame atomic absorption spectrophotometry; Gas chromatography-mass spectrometry; Heavy metals; Honey; Pesticide residue

INTRODUCTION

Honey bee honey which is syrupy and viscous substance is produced by honeybees from the nectar of flowers or from the secretion of living parts of plants, in which honeybees transform through enzymatic activity and store it in wax structures called honeycombs until maturation [1,2] should be safe for all consumers and pollinators. It has medicinal and therapeutic effects [3].

Honey has been recognized as a biological indicator of environmental pollution [4]. Determination of heavy metals in honey is of high interest mainly for quality control [5].

Pesticides is defined as a substance for destroying harmful insects or chemical or biological substances that are designed to kill or retard the growth of pests interfering with the growth of crops, desired by humans and it has benefits as well as side effects. The substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport also come under the category of pesticides [6]. There are synthetic and bio pesticides. Pesticide residues in honey can be occurred when bees in search of food, visit crops that have been treated with various agrochemicals when beekeepers use chemicals to control bee pests or diseases [7].

The study district is identified as one of the potential areas for beekeeping in Ethiopia and honey is an important source of income for small holder farmers in the area. However due to expansion of floriculture and other industrial wastes have affecting beekeeping activities that might contaminate honey and other products. Thus the area is might be exposed to pesticides that maybe released from floricultural industries and other agricultural activities. In addition, there are factories that may also release trace metals to the area. The aims of this study is to determine the concentration levels of selected trace metals in honey samples collected from the study area [8].

MATERIALS AND METHODS

Description of the study area

The study was undertaken in Walmara district of Oromia Special zone around Finfinne in central highland of Ethiopia from January 2018 to May 2018. The total land area of district is 80,927 hectares. This district is located at 25 Km to the west of Addis Ababa (8.5°-9.5°N and 38.4°-39.2°E) with altitude of 2000-3380 m. It is bordered by Finfine to the East; Ejere district to the West, Sululta district to the North, Sebeta Hawas district from the south and its weather condition is classified as 39% Woina Dega and 61% Dega. The district has 1,853 traditional, 870 transitional, 843 modern beehives. The average honey yields obtained was 20 Kg, 15 Kg and 5 Kg per hive per annual from modern, transitional and traditional, respectively. Please put the map of the study area and if possible annual average temperature and rainfall.

Sample collection, storage and pretreatment

Unprocessed representative honey subsamples, twenty in number were collected using purposive sampling technique from selected beekeepers. The samples of each weighing about 1 kg were collected during active season and preserved in clean plastic containers and transferred to laboratory.

Four samples were used to determine pesticides while 8 honey samples analyzed to determine trace metals. The samples were placed in contaminant free polyethene plastic container, labeled and stored in refrigerator at -20°C until analyses.

Equipment and reagents

The laboratory apparatus that was used during the study include: measuring cylinders, funnel, filter papers, pipettes and micropipettes, round bottom flasks, heating mantle, refrigerator and analytical balance. In addition, Agilent 7890B gas chromatograph connected to an Agilent 5977AMSD system equipped with an auto sampler G4513A, GC/MS were used for detection of pesticide residues.

All Reagents and chemicals used in the analysis were of Analytical Grade. A combination of concentrated HNO_3 and H_2O_2, H_2SO_4 were used in digestion of honey samples, blank and spiked solutions at optimum condition. Deionized water was used during the research for sample preparation, dilution and rinsing apparatus prior to analysis. Hydroquinone 1, 10 phenanthroline hydrate, sodium acetate trihydrate, ferrous ammonium sulfate hexahydrate were used for the determination of Iron metal in the honey sample.

Pesticide standards were used from JIJE Laboratory Services

in Addis Ababa in Ethiopia with purity between 98.2 and 99.5%. Acetonitrile high-performance liquid chromatography grade were used. Anhydrous magnesium sulfate, acetic acid and sodium acetate was obtained from Merck.

Sample preparation for pesticides analysis

AOAC official method 2007.01 was used for analysis of pesticide in the honey samples. In this particular method, the QuEChERS (quick, easy, cheap, efficient, rugged, safe) method were used for extraction of pesticide from honey samples using a single-step buffered acetonitrile extraction and salting out liquid–liquid partitioning from the water in the sample with $MgSO_4$ [9].

Determination of metal contents

Digestion of honey samples: In digestion of honey samples blameless optimum condition is obtained when clear solution was formed. Optimum condition was obtained by making different trials using various volumes of solvents, times and temperatures of digestion. Thus, exactly 1 g of honey sample were accurately weighed on a digital analytical balance and transferred quantitatively in to a 250 ml round bottom digestion flask. Four milliliters of HNO_3 and 3 ml of H_2O_2 were mixed and added to the weighed sample. The solvents were freshly prepared. The sample was swirled gently to homogenize the mixture. Then, it was fitted to a reflux condenser and digested continuously for three hours on a heating mantle by setting the temperature at 240°C until clear solution was obtained. Each honey sample was digested in triplicates. Thus, a total of twenty four digests were carried out for the eight honey samples. Deionized water was added to the cooled solution to dissolve the solid or semi-solid formed on cooling and to minimize dissolution of the filter paper by the digest residue while filtering with filter paper. The round bottom flasks were rinsed subsequently with deionized water in to 50 ml volumetric flasks and finally the volumetric flasks were made up to the mark with deionized water.

Reagent blanks digestion also performed for correcting the effect of the blank in parallel with the honey samples keeping all digestion parameters the same. Six reagent blank samples were prepared for the analysis of the honey samples. All the digested samples were stored in a refrigerator until analysis. Then, Ni, Cd, Cu, Zn and Pb were determined by using AAS and Fe Uv-visible spectrophotometer, respectively.

RESULTS AND DISCUSSION

Metals concentration

One of the aims of this study is to determine the concentration levels of selected trace metals in honey samples collected from the study area using FAAS was used to determine metals such as Pb, Zn, Cu, Cr, Cd and Ni whereas UV- visible spectrophotometer for Fe content of the samples. Accordingly the concentrations of the heavy metals were found to be above the detection limits in all honey samples and the concentrations of the metals were summarized in table 1 with their corresponding % RSD. Concentration of metals in honey sample from WCW1 contains high concentration of Fe, Ni and Zn, respectively.

The second honey sample analyzed was WC2 honey. Lead is poisonous metal was found to be higher than the permissible range in sample from WCW1 which needs more study in the future. As WCW1 honey mentioned above this sample also contain high concentration of Fe. The sample is also rich in essential metal Zn. Concentration of Cd was slightly higher than the permissible range in this sample Concentrations of Cu, Ni and Pb were also found to be high. The metals concentration levels from highest to lowest Fe>Zn>Pb>Cu >Ni=Cr >Cd for the sample from the site WW2.

The third study site of honey sample whose trace metals analyzed was WC3 honey. Honey sample from this site relatively shown less concentration of the metals. Non-essential metals Pb and Cd were found to be slight amount in this sample. The honey sample from this site was found to be rich in essential metals of Fe and Zn.

The fourth honey sample studied was BW4. The sample from this site had high concentration of essential metals (Fe, Zn and Ni). The order of concentration of these metals in decreasing order: Fe>Zn>Ni for essential metals and Pb>Cu>Cr>Cd for non-essential metals. The fifth honey sample studied was GL5 honey. Compared to other

Table 1: Metals concentration (µg/g) in eight honey samples (Average metals concentration ± SD and their Corresponding % RSD.

Study sites and %RSD	Heavy Metals						
	Cd	Cu	Cr	Ni	Pb	Zn	Fe
WCW1	0.13 ± 0.01	0.60 ± 0.03	0.22 ± 0.01	0.22 ± 0.02	0.90 ± 0.03	1.41 ± 0.01	8.19 ± 0.40
[a]%RSD	2.7	4.4	5.6	8.0	3.3	0.7	8.2
WC2	0.7 ± 0.01	0.45 ± 0.01	0.41 ± 0.03	0.48 ± 0.03	0.37 ± 0.02	2.46 ± 0.05	4.87 ± 1.95
[a]%RSD	2.9	2.7	7.5	6.0	6.1	2.1	4.0
WC3	0.05 ± 0.01	0.53 ± 0.02	0.41 ± 0.01	0.29 ± 0.01	0.39 ± 0.02	1.74 ± 0.03	7.08 ± 3.52
[a]%RSD	2.2	1.3	3.5	2.6	4.4	1.9	5.0
BW4	0.07 ± 0.01	0.26 ± 0.01	0.26 ± 0.02	1.22 ± 0.06	0.49 ± 0.03	1.47 ± 0.01	6.24 ± 0.78
[a]%RSD	1.4	0.8	6.7	4.6	6.1	0.4	7.3
GL5	0.04 ± 0.01	0.42 ± 0.01	0.38 ± 0.01	0.83 ± 0.03	0.65 ± 0.03	1.57 ± 0.06	6.23 ± 0.40
[a]% RSD	1.3	2.7	2.7	3.5	5.3	4.0	6.1
GS6	0.25 ± 0.01	0.44 ± 0.01	0.50 ± 0.02	0.61 ± 0.04	0.59 ± 0.01	6.94 ± 0.32	10.59 ± 2.35
[a]% RSD	4.2	3.1	3.2	7.2	2.3	4.6	3.5
AS7	0.65 ± 0.06	0.36 ± 0.01	0.16 ± 0.01	0.76 ± 0.01	0.66 ± 0.02	3.60 ± 0.05	9.57 ± 0.78
[a]%RSD	9.3	1.4	5.7	0.8	2.8	1.4	8.2
QL8	0.11 ± 0.01	0.38 ± 0.02	0.24 ± 0.01	0.40 ± 0.04	0.57 ± 0.01	3.82 ± 0.32	11.79 ± 0.39
[a]%RSD	7.0	5.0	5.2	9.4	1.2	8.3	3.2

[a]RSD is to indicate percent Relative Standard Deviation
SD is to indicate Standard Deviation
Where, WCW1, WC2, WC3, BW4, GL5, GS6, AS7 and QL8 are abbreviations of Walmara Choke one , Walmara Choke Jatani, Walmara Choke Jatani one, Burka Walmara, Gole Liben, Garasu Sida and Qarsa Lafto sampling sites of the study area, respectively.

honey samples, GL5 honey was found with the least amount of the corresponding metals except for the metals Zn and Fe.

The sixth honey sample studied was GS6 honey. Compared to other honey samples, GS6 honey shown the highest amount of the Zn metal. The honey also contains high concentration of Fe. The honey sample from AS7 study area was another target site. The sample was found with the least amount of Cu and highest Fe metals levels. The other honey sample studied was QL8 honey. Compared to other honey sample, QL8 honey was found with the highest amount of Fe metal. Even though the levels of metals concentration in each sample are similar, the metal concentration levels of each sample were found to be quite different from sample to sample and site of sampling to site of sampling.

The essential metal Fe is found to be highest followed by Zn in all samples of honey. The non-essential metals such as Cd and Pb levels are low as shown in table 2. Cd was high in two honey samples where as in the rest honey samples, it was below the maximum values allowed according to FAO and WHO. Pb metal was also found high in five honey samples but the level of Cu metal analyzed in the eight honey samples were found below maximum tolerable limits [10].

Generally, the level of this selected metals in the honey samples from district was found to be Fe>Zn>Ni>Pb>Cu=Cr>Cd from highest to lowest level of concentrations.

These findings show that there is considerable variation in metal contents among honey samples of different study sites. The data difference is most likely due to the floral type, the botanical origin, storage conditions, and anthropogenic factors.

The range of the metals were mentioned as follow: Fe was found to be highest with mean concentration ranging from Fe (4.87 to 11.79 µg/ g) followed by Zn (1.41 to 6.94 µg/ g), Cu (0.22 to-1.22 µg/ g), Pb (0.37-0.90 µg/ g), Cd (0.04 -0.70 µg/ g), Ni with mean concentration range of (0.26-0.60 µg/ g) and Cr (0.16- 0.50 µg/ g).

Comparison of present findings of metal contents of honey with reported values: There can be dissimilarity in sampling, sample preparation and analysis methods even though various chemical analysis target to a similar objective. Bearing in mind all these, the result of the present study can be compared to the findings of other study findings.

The table below shows that the concentration level of Cd in a sample in table 1 is higher than the WHO/FAO permissible levels. Samples analyzed in WCW1 and AS7 sites were higher in Pb concentration than the permissible level of WHO that may be due to cement factory find at the sampling area. The other six samples are in a good agreement with most of the results reported from different countries [10]. This need further studies on the geographical origin of the samples to aid to find out probable sources of heavy metal contamination and vegetation of the area from which the honey was originated.

Determination of pesticide residues levels

Pesticide residues analyzed in the AS, BWB, GSL and WC honey samples were aldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, P,P' DDD, P, P'DDE, P, P'DDT, O, P-DDD, endosulfan, endrine, heptachlor, heptachlor exo-epoxide and diaznone. According to this study, the residues were found to be lower than the detection limits for the honey samples analyzed.

The European regulation 396/2005 EC set the limit at 10 µg kg^{-1} for substances for which no MRL had been established. Since 1 September 2008 the European Commission has set new MRLs, which mostly are between 10 and 50 ng·g-1 in honey [15]. According to a report, of 90 analyzed samples, pesticide residue monitoring showed that 44.4% of the samples contained no detectable residues of the target pesticides [16]. Fortunately, results of this study shown that the honey samples collected from the study area were free of the organochlorine residues that studied. Therefore, the honey from the study area is safe for consumption.

Even though, the findings of the study shown that there is no significant pesticide residues in the tested honey samples, the fact no pesticides were detected or not exceeded the admitted level does not necessarily mean that farmers are not using pesticides because some time honeybees can make biological transformation/detoxification of toxic substances and extract through their feces to sustain their life.

Estimated Daily Intake (EDI) for the detected pesticide residues in the honey sample following the international guidelines [17] and [6] is determined by using the following equation:

$EDI = \Sigma C \times F / (D \times W)$ [18]

Where, C is the mean of pesticide residues concentration in honey (µg/ kg), F is mean annual intake of honey per person (2 kg per person approximately), D is number of days in a year (365), and W is mean body weight (60 kg) [19].

Since there were no detected residues in the samples, the estimated daily intakes of pesticides in the samples less than detection limit were below the Accepted Daily Intake (ADIs), which may indicate that the honey consumption has a negligible influence to health risk. If the hazard index of the pesticide residue is lower than unity, then the consumer is considered to be adequately safe. The hazard index values showed that all the intakes of pesticide residues remain clearly below the safe limit.

Table 2: Metals concentration (µg/ g) comparison in the honey samples.

Metals	Country and their reported metals level ranges						
	WHO/FAO	Brazil	Chile	Pakistan	Switzerland	Venezuela	Ethiopia
Cd	0.25						0.04-0.70
Cr			0.03-1.92		0.001-0.03		0.16-0.50
Cu	2						0.22-1.22
Fe		1.50-6.24	0.1-6.36	4.35-7.54	0.136-9.85	1.1-5.2	4.87-11.79
Ni			0.01-1.04	1.02-1.4	0.001-1.966		0.26-0.60
Pb	0.5						0.37-0.90
Zn			0.01-4.73	1.98-2.94	0.016-4.133	1.1-24.2	1.41-6.94
References	[10]	[11]	[12]	[13]	[7]	[14]	Present study

CONCLUSIONS

A good percentage recovery for metals analyzed was obtained (85-104%). The study also showed the analyzed metals were found to follow the decreasing order: Fe>Zn>Ni>Pb>Cu>Cr>Cd. Metals such as Cu, Cr, Zn and Ni are in acceptable ranges but Pb and Cd were slightly higher in WCW1, AS7 and WC2 than the permissible levels of national and international standards. Even though, the ranges of obtained parameters are in acceptable by different national and international standards, some values are slightly higher than the standards.

Pesticide residues were below detection limit. Therefore, it can be stated that the honey from the study area is safe to consumers and bees of the area. However, more study is important using more sensitive and recent analytical instruments to prove the findings.

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