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Analysis of Heavy Metals and Physicochemical Properties in Honey Along the Ndola- Lusaka Road, Zambia.

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ABSTRACT

Analysis of the concentration of selected heavy metals i.e. cobalt, copper, zinc, iron, lead, manganese, and chromium in honey samples was done in order to determine the level of heavy metals in the study area and to determine the health safety of the honey consumed. The honey samples were from Kapiri Mposhi and Kaoma districts of Zambia. Acid aqueous solution was used in the digestion of the honey samples and an Atomic Absorption Spectrophotometer was used to analyze the concentration of heavy metals. Physicochemical properties such as moisture content, Ash content and pH of the honey were also determined. Kapiri Mposhi Sample and from Kaoma sample had moisture content of 13.77% and 15%, respectively. The rest of the samples had moisture content within the world honey standard of 18% to 23%. The ash content values ranged from 2.8% to 15.7%, exceeding the maximum limit allowed of 1%. The pH values recorded were within the accepted range (3.5 –5.5) according to codex standards. The concentrations of the heavy metals in decreasing order were as follows: Zn>Fe>Cu>Mn>Pb>Cr>Co. Zinc had the highest concentration in all samples with mean concentration range of 9.68 to 19.8 mg/kg, iron (6.02 to 11.95 mg/kg), copper (1.48 to 1.95 mg/kg), manganese (0.2 to 1.57 mg/kg), lead (30.0075 to 1.23 mg/kg), chromium (<0.05 to 0.55 mg/kg) cobalt which had concentrations in all samples less than 0.05 mg/kg. All the metals were found to be below the maximum residue limits of the WHO/FAO. It was thus concluded that the honey collected was of good quality in terms of its metal content and physicochemical properties. It can also be said that the environment from which the honey samples were collected was not contaminated with toxic heavy metals.

Key words: *Heavy Metals, Honey, Atomic Absorption Spectrophotometer, Physicochemical properties.*

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Introduction

Honey is a sweet yellowish-brown fluid made by honeybees from the nectar of flowers. Honeybees gather the secretions from plant parts such as flowers and then they transform and combine it with other material, they then leave it in the honey comb to ripen (Bibi et al. 2008). Honey is used as food and as an important element in different kinds of manufactured foods. It may be an important source of vitamins and micro and macro-elements vital for human health (Formicki, et al 2013). These elements include a composite mixture of carbohydrates and other minor substances such as proteins, amino acids, organic acids, minerals and vitamins (Ahmida et al. 2013). However, honey composition depends on several factors such as floral source used to collect the nectar, geographical origin, climatic conditions and seasonality (Perna et al. 2012). Honey also acts as a therapeutic substance by possessing antibacterial, antioxidant, anti-inflammatory and anti-tumoural properties (Perna et al. 2012).

Honey can be used to collect information about the environment within the bees' exploration area. Honeybees' accretions are as a result of their interaction with air, water, flower and soil. They go from flower to flower, touch leaves and branches, drink water from various pools and their hairy bodies collect aerosol particles. Moreover, honeybees can forage on plants in an area as large as 7 square kilometers or more. For example, if it is supposed that any hive has at least 1000 worker-bees and that each of them forages on one thousand flowers per day, the honey produced daily can be considered the outcome of at least one million contacts. In this way, the forage area is efficiently sampled for trace elements (Erbilir et al, 2005). Therefore, honey is appropriate for use as a biological indicator of environmental contamination (Formicki et al 2013).

The term 'heavy metals' has no one specific definition, most researchers agree to define heavy metals as a group of elements between copper and mercury on the periodic table of the elements that have atomic weights between 63.54 and 200.590 and a specific gravity greater than 4.0 (Duruibe 2007). Living organisms require trace amounts of some heavy metals including cobalt, copper, iron, manganese, molybdenum, vanadium, strontium and zinc, but excessive levels can be harmful to the organism.

When consumed , heavy metals combine with the body's biomolecules such as proteins and enzymes to form stable bio toxic compounds, thereby disfiguring their structures and preventing their normal function (Duruibe 2007). The accumulation of metals can be as a result of external sources such as industrial pollution, improper treatment and agrochemicals. Air and soil contain

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heavy metals, mainly from industry and traffic, which can also contaminate the bee colony and its products. The hairy bodies of bees can trap heavy metals from the atmosphere and taken to the hive with pollen (Pehlivan 2015).

MATERIALS AND METHODS

Study Areas and Sampling Sites

Google Earth 2017

Figure 1: Showing map of study area

GPS coordinates

Table 1. GPS Coordinates for the sampling sites

Sampling point (code)	GPS coordinates	
Kapiri Mposhi	13˚58′ 17S	28˚40′11E
Kaoma	14˚46′59.99″S	24˚48′0.00″E

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Four honey samples were bought in the Central province, Zambia, particularly in the area known as Greenleaf which lies between Kapiri Mposhi and Kabwe. The fifth honey sample was collected in the Western province, Zambia, particularly in a town known as Kaoma. The honey samples from Central province were bought from different traders and they were packed in 500ml plastic bottles.

Chemicals and Reagents

A mixture of concentrated hydrochloric acid and nitric acid (Merck Chemicals) in the ratio 3 to 1 was used to clean the crucibles. Sodium carbonate was used to further clean the crucibles. 25% hydrochloric acid was used to extract the metals from the ash. Stock standard solutions for each metal analyzed were prepared. Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

Physicochemical Analysis of Honey Samples

Determination of the Ash content of Honey Samples

Honey normally has low ash content and this depends on the materials collected by the bees foraging on the flora (Melorose et al. 2015). The variable was calculated as follows:

Ash (% by mass) = (W1-W2)/M X 100…………..Equation 1

Where, W2=weight of empty crucible

W1=weight of the ash and crucible

M = mass of the sample taken for the test

Determination of the pH of Honey samples

The pH of honey samples was measured by using a pH-meter (HANNA).70 mL of deionized water (pH 7.0) was added to 10 g of honey and mixed thoroughly. Then after performing calibration of the instrument, the pH was measured directly.

Determination of Moisture content of Honey samples

The moisture content of each honey sample was determined by weighing out 5g of the Sample and placing it into a pre-weighed metallic drying dish. The sample was then dried to a constant weight in an oven at 105 ˚C for 4 hours and its weight taken again.

Moisture content= $\frac{M1 - M2 \times 100}{M1 - M0}$. Equation 2

Where: M0 = Weight of metallic dish

M1 = Weight of the fresh sample + dish

M2 = Weight of the dried sample + dish

Determination of Metal contents of the Honey Samples

Firstly, 5g of honey samples was taken into a crucible and placed on a hot plate to be heated slowly to completely char the honey samples. The crucible was then moved to a muffle furnace at a temperature of 450 ˚C to turn the honey into ash. When the ash was ready, Hydrochloric acid was added to the ash and the solution was evaporated to dryness. 10 ml of 25% HCL was added. The solution was then filtered and prepared up to 50 ml with HCL. An Atomic Absorption Spectrophotometer (As 200, Perkin Elmer) was used to analyze the solution for heavy metal concentration (Singh, 2014). After the instrumental operating conditions were optimized for maximum signal intensity of the instrument, the samples were analyzed in duplicates. The conditions used for analysis are shown in Table 1.

Table 2 Instrumental settings used to determine the heavy metals

	Cr	Cu	Fe	Mn	Pb
wavelength	357	324.75	372	403.08	283
Slit length	1.8/0.6	1.8/0.6	1.8/0.6	1.8/0.6	1.8/0.6
Acetylene flow	3.62	3.62	3.62	3.62	3.62
Oxidant flow	10	10	10	10	10

Recovery test

Zinc was used for spiking the sample. The procedure was as follows:

The digest (25ml) for sample 1 was analyzed for the concentration of zinc using an atomic absorption spectrophotometer. 1ml of 3mg/L standard zinc solution was then added to the 25ml digest. The solution was then analyzed for the concentration of zinc. The percentage recovery was calculated as follows:

Percentage recovery= spike result divided by expected result x spike volume over original volume x 100%. …………..equation 3

Where expected spike result = sample result + standard concentration

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RESULTS

Table 3: Results for the moisture content

Sample number	Weight of metallic dish M0 (g)	Weight of fresh sample + dish M1 (g)	Weight of dried sample + dish M2 (g)	Moisture content (M1-M2)/(M1-M0) (%w/w)
1	15.66	20.66	19.66	20
2	15.54	20.55	19.86	13.77
3	15.75	20.75	19.79	19.2
4	15.58	20.58	19.68	18
5	12.78	17.78	17.03	15

TABLE 4 Results for the ash content in honey samples

Sample Number	Weight of empty crucible (W2) g	Mass of sample being tested (M) g	Weight of the ash and crucible (W1) g	Ash(%by mass) (W1-W2)/M x 100
1	25.09	5.00	25.23	2.8
2	24.11	4.99	24.29	3.6
3	27.30	4.96	28.08	15.7
4	21.23	4.99	21.44	4.2
5	39.41	5.01	40.15	14.8

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Table 5 Results for the pH of honey samples

SAMPLE NUMBER	pH READING
1	4.97
2	4.59
3	4.80
4	4.57
5	3.91

Table 6 Concentrations of Heavy metals in the honey samples (mg/kg)

metals	Average concentrations of metals ± SD				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
copper	1.475 ± 0.53	1.71± 0.03	1.95± 0.08	1.5± 0.59	1.9± 0.27
cobalt	<0.05	<0.05	<0.05	<0.05	<0.05
manganese	1.57±0.59	0.685±0.11	0.2±0.195	1.33±0.73	0.32±0.31
iron	8.59±0.96	6.02±3.49	8.7±1.95	10.15±2.95	11.95±6.75
zinc	9.68±2.39	13.25±3.35	11.05±3.25	19.8±1.93	14.4±6.75
lead	0.01±0.005	0.01±0.005	0.0075±0.0025	0.0775±0.0725	1.23±1.22
chromium	0.545±0.33	0.065±0.06	<0.05	<0.05	<0.05

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Table 7 Recovery test result

Metal	Standard Concentration mg/L	Concentration in sample mg/L	Spike Concentration mg/L	Expected Spike result mg/L	Initial Volume ml	Final Volume ml	Recovery (%)
zinc	3	1.46	3.71	4.46	25	26	86.5

Discussion

The moisture content is important to consider when looking at the honey quality, because this parameter is related to honey spoilage through fermentation (Melorose *et al*. 2015). The present study shows variations falling between 13.77% and 20% as shown in Table 2. This difference may be accounted for differences in; botanical origins, weather conditions, original moisture contents of the nectar, harvesting season, the conditions of harvesting, transportation and storage of honey samples and the degree of maturity (Melorose *et al*. 2015). According to the world honey standards, the standard moisture content lies between 18% and 23%. In this study, sample 2 from Kapiri Mposhi had moisture content of 13.77% and sample 5 from Kaoma had moisture content of 15%. Both of these samples had moisture content below the world honey standard of 18% to 23% but the rest lay within this standard. The low water content of some samples could be the result of processing or even storage(Melorose *et a*l. 2015).

The ash content in honey is generally low and influenced by the chemical composition of nectar that varies according to the different botanical sources involved in honey formation. It can vary between 0.02% and 1.0 % and the maximum limit allowed by legislation for honey from floral sources is 1% (Melorose *et al,*. 2015). However, the findings of this study exceeded the limit. The ash content values ranged between 2.8% and 15.7%, with no values below 1%. In other studies like the one undertaken in Turkey (Erbilir & Ozlem, 2005) the ash content was found to be 0.05-0.56% which are lower compared to what was obtained in this study but their result was in conformity with the Turkish Alimentarus Codex .This could imply that there is need to have a standard for the honey that is made in Zambia in regards to such parameters .

Generally, honey is acidic and the pH values lie between 3.5 and 5.5, due to organic acids that contribute to honey flavor and stability against microbial spoilage .The pH of the honey can indicate microbial contamination and adulteration (Melorose *et al*. 2015). The pH values of all honey samples were found to be acidic. The pH values recorded were: 4.97, 4.59, 4.80, 4.57,

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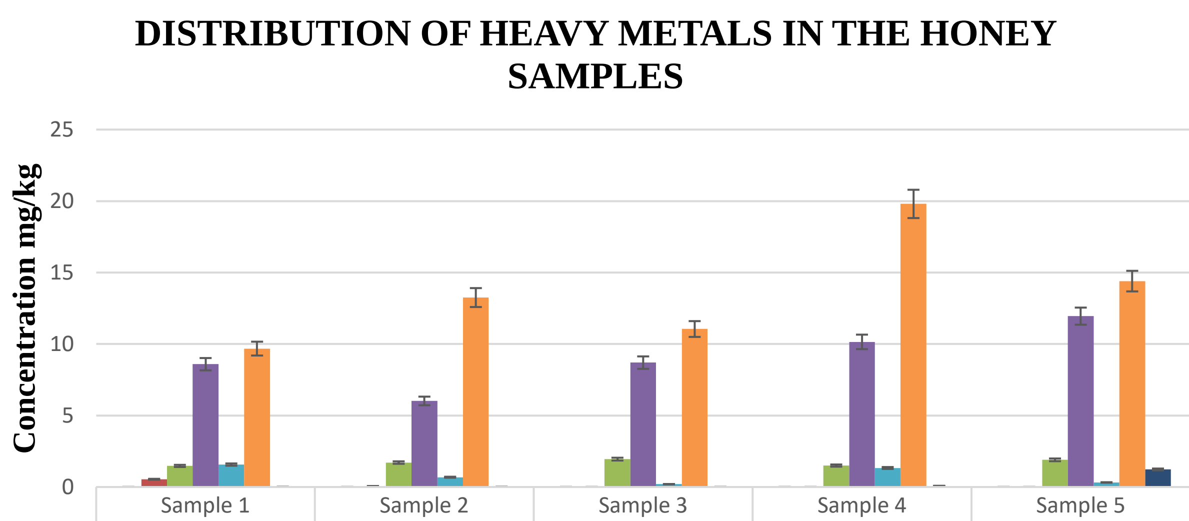
and 3.91. The pH was also within the accepted range (3.5 –5.5) according to codex standards.

Distribution of metals in honey Samples

Each of the honey samples analyzed contained different concentrations of heavy metals. It was observed that the average concentration of Zinc was the highest followed by iron, copper, manganese, lead, chromium and cobalt in that order. Figure 2 below shows this distribution



	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Co	0,005	0,005	0,005	0,005	0,005
Cr	0,545	0,065	0,005	0,005	0,005
Cu	1,475	1,71	1,95	1,5	1,9
Fe	8,59	6,02	8,7	10,15	11,95
Mn	1,57	0,685	0,2	1,33	0,32
Zn	9,68	13,25	11,05	19,8	14,4
Pb	0,01	0,01	0,0075	0,0775	1,23

Figure 2 the distribution of heavy metals in the honey samples

Sample number 1 from Kapiri Mposhi contained zinc as its highest concentration (9.68±2.39mg/kg) followed by iron (8.59±0.96mg/kg), manganese (1.57±0.59mg/kg), copper (1.475 ± 0.53 mg/kg), chromium (0.545±0.33mg/kg), lead (0.545±0.33 mg/kg) and cobalt (<0.05mg/kg) in that order.

For sample number 2, the concentration of zinc was the highest (13.25±3.35mg/kg) followed by iron (6.02±3.49mg/kg), copper (1.71± 0.03 mg/kg), manganese (0.685±0.11 mg/kg), chromium (0.065±0.06 mg/kg), lead (0.01±0.005mg/kg), cobalt (<0.05mg/kg) in that order.

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The distribution of heavy metals in sample 3 in decreasing order was as follows: zinc (11.05±3.25mg/kg), iron (8.7±1.95mg/kg), copper (1.95± 0.08mg/kg), manganese (0.2±0.195mg/kg), lead (0.0075±0.0025mg/kg), for both chromium and cobalt (<0.05mg/kg). The distribution of heavy metals in sample 4 in decreasing order was as follows: zinc (19.8±1.93mg/kg), iron (10.15±2.95mg/kg), copper (1.5± 0.59mg/kg), manganese (1.33±0.73mg/kg), lead (0.0775±0.0725mg/kg), for both chromium and cobalt (<0.05mg/kg). The distribution of heavy metals in sample 5 in decreasing order was as follows: zinc (14.4±6.75mg/kg), iron (11.95±6.75mg/kg), copper (1.9± 0.27mg/kg), Lead (1.23±1.22mg/kg), manganese (0.32±0.31mg/kg) for both chromium and cobalt (<0.05mg/kg).

Many heavy metals when consumed in the right amounts are important for biological systems. However, heavy metals have harmful effects when their concentration exceeds maximum allowed quantities. For example, excess of iron in food is associated with degenerative brain disease, such as Parkinson and Alzheimer. A higher concentration of zinc in human diet can interfere with white blood cells and other defense systems against infections and cancers. An increase of the doses of manganese in food can produce nervous system disturbances (Dima, 2012). According to WHO/FAO standards on heavy metals in food, all the samples had concentration levels below the maximum allowed. The levels should not exceed 60 mg/kg for zinc, 30 mg/kg for copper, 2 mg/kg for lead, and 48 mg/kg for iron. In as much as the quality control aspect of honey is important, the determination of the heavy metals can be used as an indicator of environmental contamination (Erbilir, & Ozlem,2005).

CONCLUSION

From the study no heavy metal contamination was detected as all the values obtained were below the WHO/FAO maximum residue limits. Therefore, the honey could be considered safe for human consumption. For moisture content, the honey samples did not exceed the world honey standards of 18%-23%. The pH of the honey was also within the acceptable range according to codex standards of 3.5 to 5.5. The ash content was however above the acceptable standards in all samples. It is therefore recommended that the honey from the study area is safe for human consumption and that the study area is safe for beekeeping and honey production.

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