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Determination of some Heavy Metals and Selected Phenolic Compounds in Commercial Honeys obtained from Northwestern States of Nigeria

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ABSTRACT

Natural honey is one of the highly needed products because of its exclusive, high nutritive and medicinal properties, it is among the most adulterated products globally. Honey adulteration is a global concern, it has negative effects on the nutrition and health of consumers and has become a common practice because of the high demand and limited availability of the product. This research aims to evaluate the heavy metals concentrations and the phenolic compounds in honey samples obtained from local sellers and compare the levels with honeys from credible beekeepers in Northwestern states of Nigeria (Kano, Kaduna, Jigawa, Katsina, Sokoto, Zamfara and Kebbi) in order to detect possible adulteration of the products. Results indicated that most of the heavy metals detected Cd (ND-0.10±0.16 µg/g), Pb (0.20±0.11-1.62±1.60 µg/g), Ni (0.03±0.04-0.53±0.46 µg/g), Cu (0.47±0.23-2.61±2.11 µg/g), Zn (5.75±4.04-97.63±131.74 µg/g) and Fe (6.74±1.92-61.43±98.12 µg/g) in honeys from local sellers were above the permissible range set by the International Honey Commission (IHC) and NAFDAC while the levels in samples obtained directly from beekeepers closely complied with the standards. The non-compliance of the metal levels with IHC standards in locally sold honeys could possibly be due to adulteration of the products using metal-contaminated adulterants. However, lower values were recorded for Phenolic compounds: flavonoids (154.5-521.7 mg/kg) and Phenolic acids (399.6-1075.0 mg/kg) in most of the honeys from local sellers except in honeys from Zamfara state. Generally, results from this study indicate that large percentage of the honey products sold locally in the Northwest Nigeria are suspected to be adulterated mostly with sweeteners while honeys obtained directly from beekeepers were found to agree with IHC standards. It is therefore recommended that pure honeys should be obtained directly from credible beekeepers.

Keywords: Adulteration, Flavonoids, Heavy Metals, Honeys, Phenolic Acids

INTRODUCTION

Honey produced by the honey bee is a natural super saturated sugar solution which has been seen as a highly nutritive food and is composed of a complex mixture of carbohydrates, minerals, vitamins, aromatic compounds, flavouring and enzymes with the water content of about 17 – 20% (Haouam *et al.,* 2016). The major composition of honey are carbohydrates and water (Agbajor and Otache, 2020). It is a high-energy carbohydrate food as the honey sugars are easily digestible as those in many fruits (El-Sohaimy *et al.,* 2015).

Honey is one of the major bee products, a semi liquid, sweet and flavored food stuff produced from nectar of nectarines of flowers, or secretion of plant-sucking insects which the bees collect, transform by addition of specific substances of their own, deposit, dehydrate, store and leave in honeycomb to ripen and mature (Majewska *et al.,* 2019). Natural honey is a liquid mentioned in all religious books, and accepted by all generations, traditions and civilizations, both ancient and modern. It is one of the products most widely sorted for due to its unique nutritional and medicinal properties.

Honey contains a variety of macro and micro minerals that are the minor constituents of honey present in the range of 0.02–2.03%. Honey contains high levels of potassium, sodium, calcium and magnesium, the sources of which were shown to be pollen and nectar (Altun *et al.,* 2017; Batista *et al.,* 2012). Apart from the mentioned minerals, honey may contain Fe, Cr, Se, Cu, Mn, Zn, Al, Pb, As, Cd and Hg, depending mainly on environmental factors (Mutlu *et al.,* 2017). Trace elements are mainly the ash content of honey (Altun *et al.,* 2017).

The presence of metals in honey has the potential to threaten the health of humans as consumers. Lead, arsenic, cadmium and mercury are among the most abundant heavy metals and are particularly toxic. The excessive amount of these metals in food is associated with etiology of a number of diseases especially with cardiovascular, kidney, respiratory, nervous and as well as bone diseases (Ernest *et al.,* 2018).

Honey also contains phytochemicals such as flavonoids and other polyphenols that makes it a potential functional ingredient and an antibacterial agent (Ndife *et al.,* 2014). Of the polyphenols, phenolic acids are likely to be the major group in honey (Chua *et al.,* 2013). It is well known as a natural dietary antioxidant. The components responsible for the redox properties of honeys are likely to be phenolic acids, flavonoids, vitamins and enzymes, as well as small amount of mineral content, particularly copper and iron (Gul and Pehlivan, 2018). Interestingly, they have been given considerable attention to be an eligible parameter for honey quality assessment (Lewoyehu *et al.,* 2019). The use of natural honey as food and medicine by mankind has been in existence since ancient times.

Raw honey is the most ancient sweetener, and it was noted to have been in use. The components responsible for the redox properties of honeys are likely to be phenolic acids, flavonoids, vitamins and enzymes, as well as small amount of mineral content, particularly copper and iron (Chua *et al.*, 2013). Interestingly, they have been given considerable attention to be an eligible parameter for honey quality assessment (Lewoyehu *et al*., 2019). Raw honey contains an enzyme called glucose oxidase which combines with water to produce hydrogen peroxide (H_2O_2), a mild antiseptic substance (Ndife *et al.,* 2014). Honey also contains phytochemicals such as flavonoids and other polyphenols that makes it a potential functional ingredient and as an antibacterial agent (Ramanauskiene *et al.,* 2012). of the polyphenols, phenolic acids are likely to be the major group in honey, they are well known as a natural dietary antioxidant (Chua *et al.,* 2013). throughout the world several million years ago (Eteraf-Oskouei and Najafi, 2012).

MATERIALS AND METHODS

The materials used and methods adopted in this research are as described: Pure standards of Rutin (RUE), Gallic acid (GAE) and Folin-Ciocalteu were purchased from Sigma-Aldrich Chemical Company (Steinheim, Germany). Similarly, HNO_3 and $NaNO_3$ reagents were sourced from Sigma-Aldrich. All other materials and solvents used were of analytical grade.

Sampling

One hundred and five (105) honey samples were randomly purchased from open markets within Northwestern states (Kano, Jigawa, Kaduna, Katsina, Sokoto, Zamfara and Kebbi), Nigeria. Each state was divided into three senatorial districts: Central, North and South. Five samples (control sample inclusive) were collected from each district making a total of 15 samples from each state. The samples from central senatorial districts in Kano state were labeled as KN CI, KN C2, KN C3 and KN C4, while the control sample was designated KN CC. Also, the samples from Kano North were labeled as KN N1, KN N2, KN N3 and KN N4 with the control sample as KN NC. Likewise, samples from south districts were labelled as KN SI, KN S2, KN S3 and KN S4, while the control sample as KN SC. This identification trend was used in all other States. The honey samples were obtained commercially while the pure honey samples used as control were obtained directly from bee keepers in each State. All the samples were collected in sterile containers, labeled and stored in a refrigerator in airtight plastic containers until analysis.

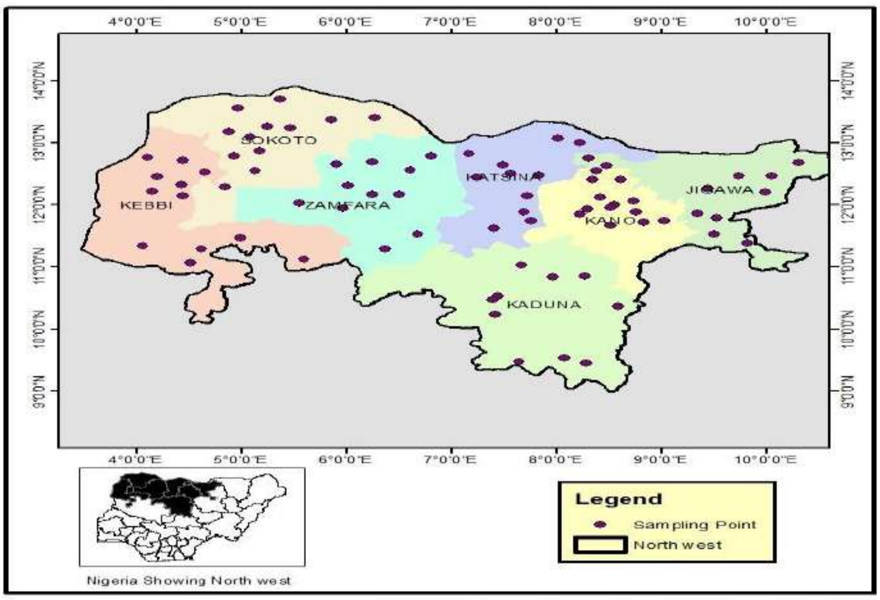

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Fig. 1: Map of the Sampling Sites

METHODOLOGY

Extract of Hoey Sample

The honey sample (6 cm^3) was dissolved in 2 cm^3 methanol and made up to 60 cm^3 with deionized water and left overnight. The mixture was filtered using Whatmann filter paper and stored in a refrigerator until analysis

Digestion Procedure

Honey sample (5 g) was weighed into a clean pre-weighed porcelain crucible. It was placed on a hot plate and heated at 300ºC until sample was dried in order to prevent loss by foaming. It was then ashed to constant weight in a muffle furnace at a temperature of 550°C. Constant weight was obtained by continually heating the sample, cooling in a desiccator and weighing at 2–3 hours intervals for about 8 hours until no change in weight was observed. The ash obtained was then dissolved in 50 cm^3 of 0.5M HNO_3. The same procedure was carried out for all the samples. All the digests and sample blank were analysed for Zn, Cd, Fe, Cu, Ni and Pb using Microwave Plasma Atomic Emission Spectrophotometer (The agilent 4210).

Determination of Total Flavonoid Content

The total flavonoid content in each sample was measured using the colorimetric assay. Honey extract (1 cm^3) was mixed with 4 cm^3 of deionized water. Then 0.3 cm^3 of $NaNO_2$ (5% w/v) was added. After five minute, 0.3 cm^3 of $AlCl_3$ (10% w/v) was added followed by 2 cm^3 of NaOH (1M) and left for 6 min. The volume was made up to 10 cm^3 with deionized water. The mixture was vigorously shaken to ensure adequate mixing and the absorbance was read at 510 nm using UV/VIS Spectrophotometer. A calibration curve (Fig. 9) was created using a standard solution of Rutin 20; 40; 60; 80; 100;120;140; 160; 180 and 200 µg/ml. The results were expressed as mg Rutin equivalents (RUE) per kg of honey (Abu-Bakar *et al.,* 2017).

Determination of Total Phenolic Acid Content

The concentration of phenolic in honey samples was estimated using modified spectrophotometric Folin-Ciocalteu method. Honey extract (1 cm^3) was mixed with 1 cm^3 of Folin-Ciocalteu reagent (1:1). After 3 min, 1 cm^3 of 10% Na_2CO_3 solution was added to the mixture and made to 10 cm^3 with deionized water. The reaction was kept in the dark for 15 min, after which the absorbance was read at 725 nm using UV/VIS Spectrophotometer. Gallic acid was used to plot a standard calibration curve at 20, 40, 60, 80; 100; 120; 140; 160; 180; 200 and 220 µg/ml (Fig. 8). The results were expressed as mg Gallic acid equivalent (GAEs) per kg honey (Abu-Bakar *et al.,* 2017).

RESULTS AND DISCUSSION

Statistical Analysis

All the tests were done in triplicate and the data were expressed as mean ± standard deviation (SD). Statistical significance of differences was determined using a one-way Analysis of Variance (ANOVA) and the Duncan Multiple range test with significant level at 95% ($P<0.05$) were considered significant.

Heavy Metals Concentration in Honey Samples

The mean concentration of heavy metals varied significantly across most of the studied states with no specific pattern and were in decreasing order of Zn > Fe > Cu > Pb > Ni > Cd.

The mean cadmium concentrations in all the studied honey samples obtained from all study areas ranged from ND µg/g in honey samples from source to the highest 0.10 µg/g in honey samples from Kano and Katsina (Fig. 2). The concentrations of cadmium (0.10, 0.10, 0.07 µg/g) in honey samples collected from Kano, Katsina and Kaduna respectively had exceeded the permissible limit of 0.05 µg/g set by World Health Organization (WHO, 2015) and were significantly higher ($p<0.05$) compared with honey samples obtained from source (control) and other states. The high concentration of cadmium might be attributed to the high traffic emissions, poor sewage system and use of fertilizers. Cadmium is released into the environment through its use in various industrial processes, and enters the food chain from uptake by plants from contaminated soil or water. Therefore, the cadmium concentration in various places depends on many variables, leading to its different concentration in honey samples in the studied place. The mean concentration of cadmium in the present study was comparable with < 0.3 µg/g reported by Chukwujindu *et al*. (2015) in honey consumed in Nigeria, 0.07 – 0.1 µg/g in honeys from Brinin-Gwari, Nigeria (Idoko *et al*., 2018), 0.028 – 0.07 µg/g in some states in Northern part of Nigeria (Odoh *et al*., 2015), 0.001 – 0.1 µg/g in and around the university of Ilorin Environ., Kwara State, Nigeria (Okeola *et al.,* 2020) and 0.02 – 0.05 µg/g from selected villages in five Local Government areas of Adamawa state, Nigeria (Toma *et al.,* 2020). Cadmium concentrations in this study were lower than 0.05 – 0.76 µg/g from different places in Karnataka (Singh *et al*., 2014).

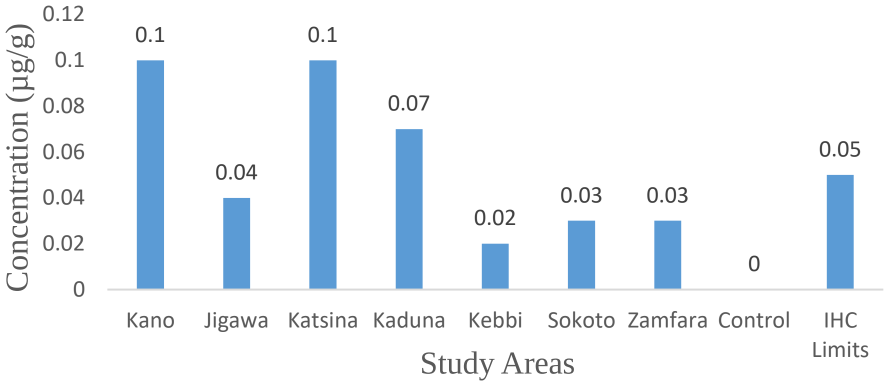
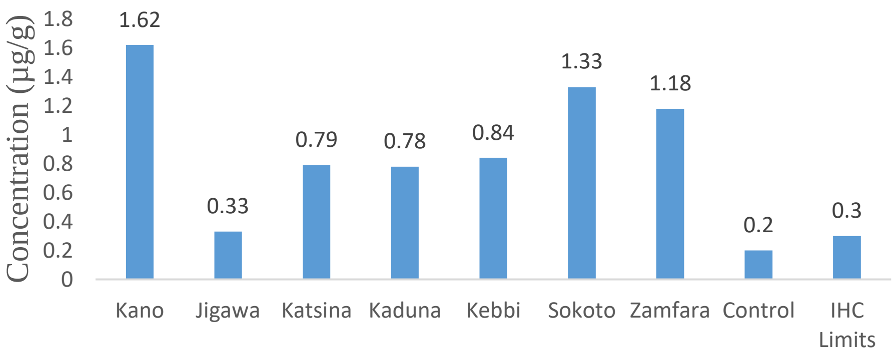


Fig 2: Mean Concentration of Cd (µg/g)

The mean concentration of lead in honey samples obtained from the study areas varied from the lowest of 0.20 µg/g in control samples from source to the highest of 1.62 µg/g in honey samples from Kano (Fig. 3). The mean concentrations of lead in all the honey samples obtained from all the study areas have exceeded the permissible limit of 0.5 µg/g set by WHO (2015) with the exception of samples from source (0.20 µg/g) and Jigawa state (0.30 µg/g). The mean concentration of lead in honey samples collected from Kano, Sokoto, and Zamfara study areas differed significantly ($p<0.05$) with the control (0.20 µg/g). More so, the maximum lead concentration in honey samples obtained from Kano was significantly higher ($p<0.05$) compared to mean concentrations in honey samples collected from all the study areas. The possible source of high lead concentration in these honey samples may be associated with vehicular emission and illegal lead mining in those regions. Lead is one of the most widespread metal pollutants that can reach human system through air, water and food. This metal has no beneficial role in human metabolism and produces a progressive toxicity and can cause health disorders (Singh *et al.*, 2014). The mean concentration of lead in the analyzed samples is in agreement with 0.8 – 1.2 µg/g reported by Mahmoudi *et al.* (2015*)* in honey from North-Western region of Iran. It is however higher than 0.041 – 0.087 µg/g previously reported for honey from some of the states in Northern part of Nigeria (Odoh *et al.*, 2015), 0.175 – 0.35 µg/g in honey produced within Nsukka and Enugu metropolis (Ernest *et al.*, 2018). Similarly, it is lower than 1.72 – 2.97 µg/g for honeys from Brinin Gwari, Nigeria (Idoko *et al.*, 2018), 0.23 – 2.53 µg/g in honey from different regions of Ethiopia (Esubalew *et al.*, 2020), 0.28 – 4.93 µg/g in honey consumed in Nigeria (Chukwujindu *et al.*, 2015) and 0.2 – 4.2 µg/g for honeys from different places in Karnataka (Singh *et al.*, 2014).



Study Areas

Fig 3: Mean Concentration of Pb (µg/g)

Nickel (Ni) was detected in all the samples of honey analyzed. The mean concentration of nickel in honey samples obtained from the study areas ranged from the minimum of 0.03 µg/g in honey samples from Jigawa to maximum of 0.53 µg/g in honey samples from Kaduna (Fig. 4). The concentration of nickel in honey samples obtained from all the study areas were higher than the permissible limits set by WHO (2015) of 0.25 µg/g with the exception of samples obtained from Jigawa (0.03 µg/g), Katsina (0.06 µg/g) and source (0.17 µg/g). The mean concentrations of nickel in honey samples across most of the study areas varied significantly ($p<0.05$), however nickel concentrations in honey samples (0.53, 0.51, and 0.51 µg/g) from Kaduna, Kano and Zamfara respectively, were significantly higher compared with concentration in honey samples from source (control). Nickel originates most of the times from the combustion of fossil fuels, source of the emission of ultrafine metal-containing particles. These airborne particles eventually deposit on vegetation, soil or surface water where bees take up heavy metals from the environment (Costa *et al.*, 2019). A wide concentration range of nickel in honey samples has been reported in the literature (Chukwujindu *et al.*, 2015). The mean concentrations of nickel in the present work are similar to 0.25 – 0.56 µg/g reported by Tutun *et al.* (2019) but were however, lower than 0.25 – 6.98 µg/g and 1. 36 – 3.92 µg/g previously reported by Chukwujindu *et al.* (2015) and Salihaj and Bani (2017) respectively

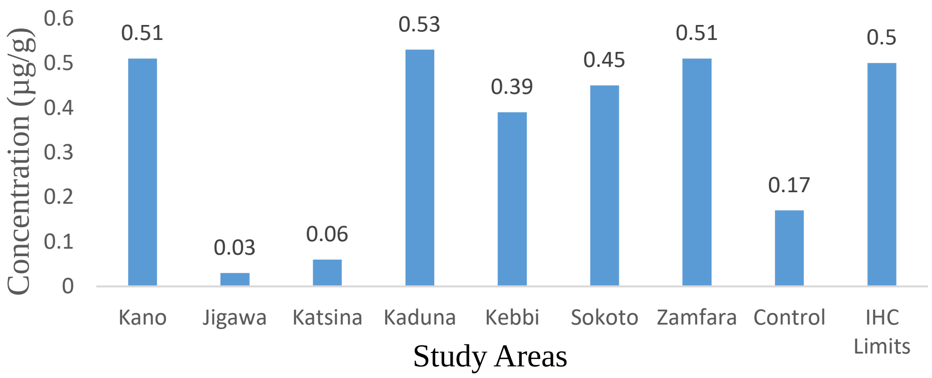


Fig 4: Mean Concentration of Ni (µg/g)

The mean concentration of copper in all honey samples analyzed ranged from the lowest of 0.47 µg/g in honey samples from Jigawa to the highest of 2.61 µg/g in honey samples from Kano (Fig. 5). The mean concentration of copper in all honey samples obtained in this study were below the permissible limits set by WHO (2015) of 5.0 µg/g. The mean concentration of copper in honey samples (2.61, 2.26, 2.46 µg/g) from Kano, Katsina, Kebbi were significantly ($p<0.05$) higher compared to honey samples collected from source (control) and all the study areas. The presence of heavy metals in soils is not only due to external contamination, but can also be of geochemical in origin. Indeed, high copper contents can occur due to mixed causes, such as abnormal native geochemical contents being complemented by mining contaminants. Copper is a vital element to the health of all living things and in humans. However, too much ingestion of copper can lead to adverse health effects in the body (Aghamirlou *et al.*, 2015). The results obtained are found to be consistent with 0.72–2.57 µg/g reported by Njokuocha *et al.* (2019) for honeys from different locations in Nigeria, 0.31–2.482 µg/g reported by Chuleeporn *et al.* (2018) for honeys obtained from different regions of Thailand and 1.85–2.35 µg/g) revealed by Beshaw *et al.* (2022). Esubalew *et al.* (2020) and Stecka *et al.* (2014) reported lower concentrations to those obtained in the present study, 0.02–1.15 µg/g and 0.01–1.42 µg/g for content of copper in honey respectively. Higher Cu concentrations (0.95–9.24 µg/g and 0.25–71.25 µg/g) were recorded by Chukwujindi *et al.* (2015) and Iwegbue *et al.* (2015) respectively in honey consumed in Nigeria and several times higher than the level noted in honey varieties produced in Turkey (0.223–198.361 µg/g) (Altunatmaz *et al.*,2018).

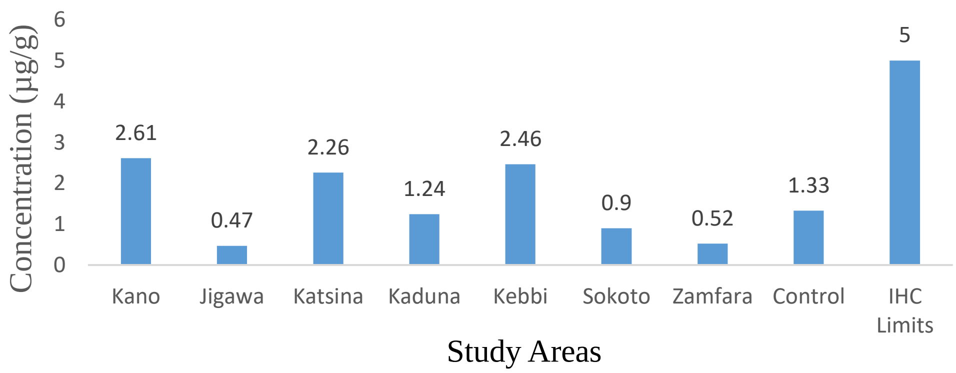


Fig 5: Mean Concentration of Cu (µg/g)

The mean concentration of zinc in all honey samples analyzed ranged from the lowest 5.75 µg/g in honey samples from Kebbi to the maximum of 97.63 µg/g in honey samples from Katsina (Fig. 6). The mean concentration of zinc in honey samples from Kano (12.33 µg/g), Jigawa (19.78 µg/g), Katsina (97.63 µg/g) and Sokoto (11.04 µg/g) obtained in this study had exceeded the permissible limits of 10 µg/g set by WHO (2015). The mean concentration of zinc (97.63 µg/g) in honey samples from Katsina was significantly ($p<0.05$) higher compared with the concentration of honey samples from the source and all the study areas. Usually, the use of galvanized containers is the most prominent source of contamination of honey as reported by Esubalew *et al.* (2020) and this could be the reason why Katsina honey that is sometimes sold in such containers are highly contaminated with zinc possibly due to leaching. Some researchers have expressed that diverse metal concentrations in honeys is extremely reliant on the kind of flowers utilized by bees and it can be the chief source of Zn contamination (Aghamirlou *et al.*, 2015). Although, zinc is an essential element for human body, high Zn intake may lead to adverse health effects. Zinc is an essential element for the organism. It plays a critical role for the structural and functional integrity of cells. It has functions in gene expression and growth. It protects from ultraviolet radiation, facilitates wound healing, contributes to immune and reduces the risk of cancer and cardiovascular disease. In most cases excess zinc generates reactive oxygen species and/or displaces other metals from active sites in proteins (Marschner, 2012). The most important sources of anthropogenic zinc in soil come from discharges of smelter slags and wastes, mine tailings and the use of galvanized containers that contain zinc (Aghamirlou *et al.*, 2015). The average Zn concentration was higher than those found in Nigerian honeys, (1.0 – 31.0 µg/g, 2.12 – 3.56 µg/g, 1.77 – 3.99 µg/g and 6.62 µg/g) reported by Iwegbu *et al.* (2015), Toma *et al.* (2020), Okeola *et al.* (2020) and Idoko *et al.* (2018) respectively. Other reported levels of Zn in honeys across the world include those from Ethiopia (9.96 – 16.03 µg/g and 1.98 – 2.04 µg/g) reported by Esubalew *et al.* (2020) and Tibebe *et al.* (2022) respectively, 0.12 – 6.63 µg/g from Iran (Aghamirlou, 2015) and 4.70 – 173.77 µg/g from Malaysia (Moniruzzaman *et al.*, 2014).

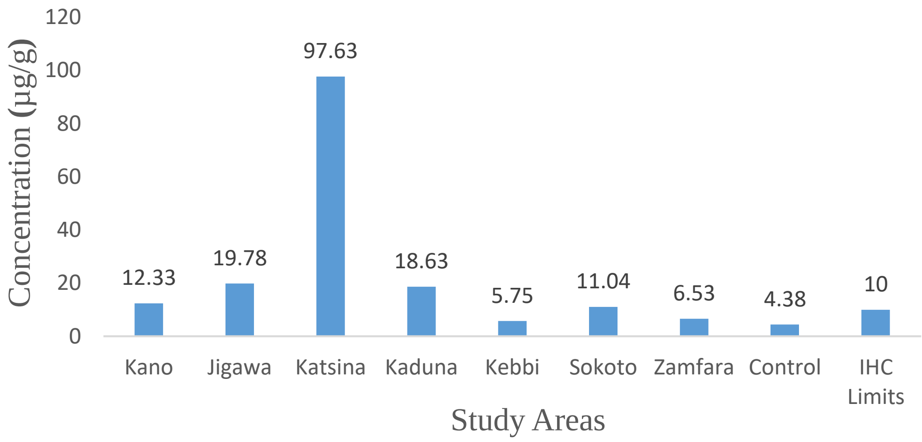


Fig 6: Mean Concentration of Zn (µg/g)

The mean concentration of iron in all the honey samples analyzed ranged from 6.74 µg/g in honey samples from Jigawa to 61.43 µg/g in honey samples from Kano (Fig. 7). Mean concentration of iron in all the honey samples obtained in this study had exceeded the permissible limit set by World Health Organization, WHO (2015) of 15 µg/g except in honey samples from Jigawa (6.74 µg/g), Katsina (13.10 µg/g) and source (12.05 µg/g). The mean concentration of iron (61.43 µg/g) in honey samples from Kano was significantly ($p<0.05$) higher compared with the concentration of honey samples from the source and all the study areas. A wide concentration range of Fe in honey samples has been reported in literature and the concentrations of Fe in our samples (6.74 – 59.76 µg/g) were comparable with Fe concentrations (ND – 40.77 µg/g) reported by Njokuocha *et al.* (2019) in honey from different locations in Nigeria and higher than Fe concentrations reported in honey samples from Ethiopia, 0.56 – 18.69 µg/g (Melaku and Tefera, 2022), Algeria, 4.22 – 8.96 µg/g (Chafik and Adnene, 2022) , Saudi Arabia, 2.08 – 8.79 µg/g (Aljedani, 2022) and Brazil, 0.12 – 8.76 µg/g (Maria *et al.*, 2013). The Fe concentrations of the study were lower than the previously detected Fe concentrations in honey samples from Nigeria, 5.0 – 163.2 µg/g (Iwegbue *et al.*, 2015) and Turkey, 3.506 – 1278.779 µg/g (Altunatmaz *et al.*, 2018). Fe is an essential element for the production of red blood cells. It has an ability to mediate electron transfer in the catalysis of enzymatic reactions which is also potentially toxic because it can catalyse the conversion of hydrogen into free radicals (Altun *et al.*, 2017).

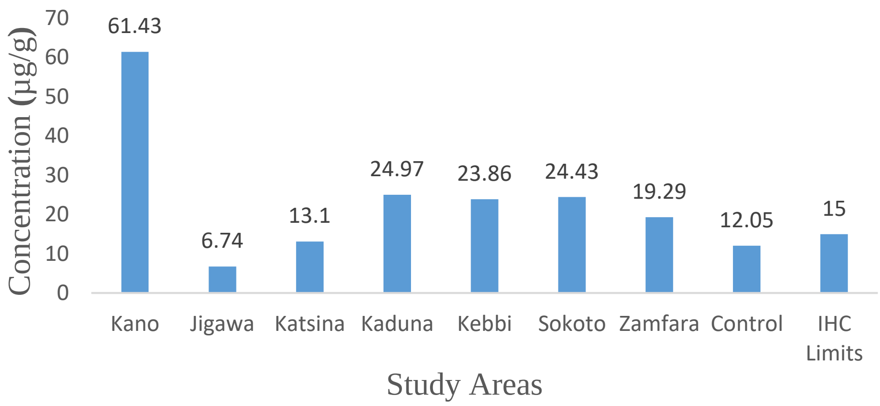


Fig 7: Mean Concentration of Fe (µg/g)

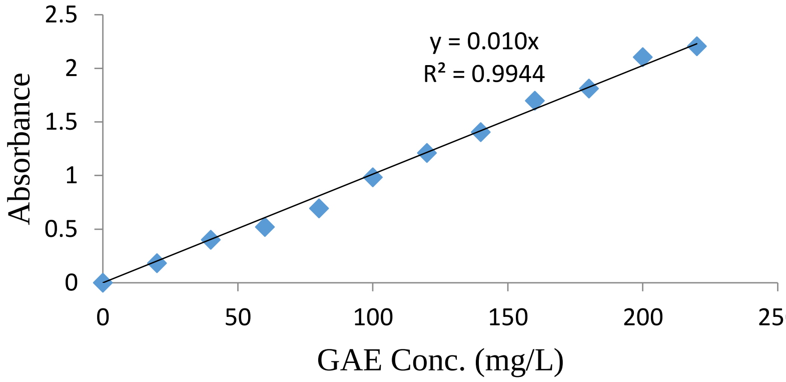


Fig.8: Calibration Curve for Gallic Acid Equivalent (GAE).

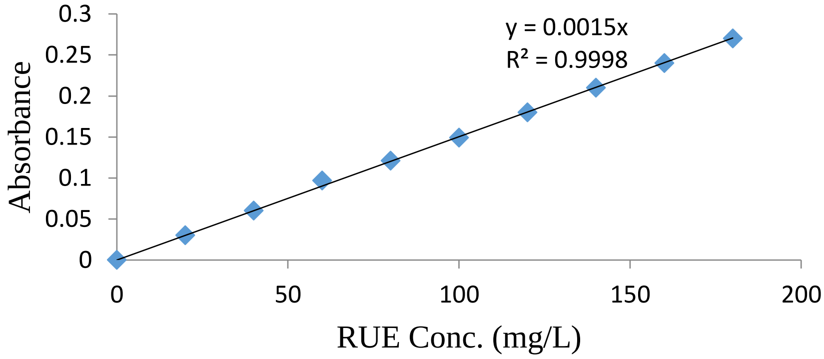


Fig 9: Calibration Curve for Rutin Equivalent (RUE).

Using the calibration plot of RUE, the flavonoid contents (mg/kg of honey) were determined for all the samples and found to be in the range of 154.5 in samples from Kaduna to 294.4 mg RUE/kg in samples from Zamfara states, while the control samples contain the highest mean value of 521.7 mg RUE/kg (Fig. 10). The average quantity for total flavonoids content in the honey samples were found to be lower than 4052 to 9661 mg/kg in honey from Southern Nigeria (Ukom *et al.*, 2019) and relatively higher than 25.2 to 272.1 mg/kg in honey from the Southern rain forest and Northern savannah ecosystem in Nigeria (Ita, 2011). In addition, some previous studies have also found the following total flavonoid content (426.0 to 9504.1 mg/kg) in various honeys from Algeria (Bakchiche *et al.*, 2020). Pham-Nhut *et al.* (2022) reported 49 to 89 mg QE/kg) from different botanical and geographical sources, 416.7 to 12497.4 mg/kg was reported by Kaya and Yildrim (2021) of honey of five different regions of Bingol province, 180 to 422 mg/kg by Sime *et al.* (2015) of natural honeys from different geographical regions of Ethiopia and 656.5 mg/kg by Moniruzzaman *et al.* (2013) of Malaysian honeys.

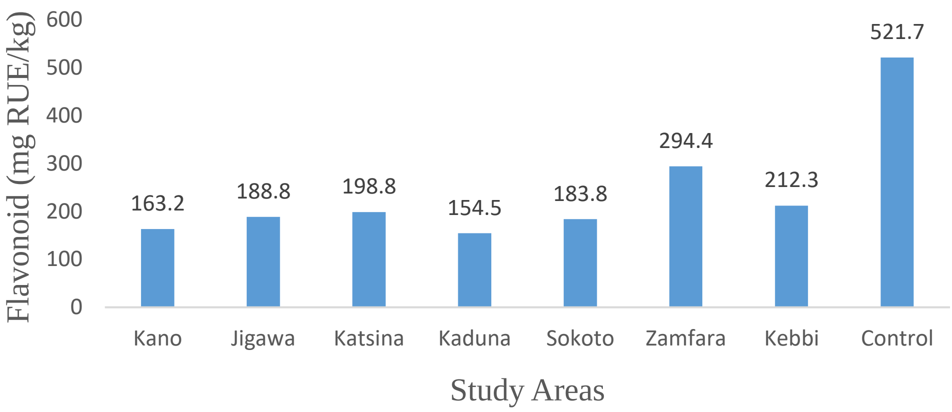


Fig 10: Variation of Mean Concentration of Flavonoid (mg/kg) in Honey Samples Analyzed

The total phenolic compound varied from 399.6 mgGAE/kg in samples from Kaduna state to 1075.0 mgGAE/kg in samples from Zamfara with an average value of 1580.0 mg/kg in honey samples from source (control) (Fig. 11). The mean concentrations of (399.6, 431.5, 433.1, 457.2 and 461.1 mg GAE/kg) in honey samples obtained from Kaduna, Sokoto, Jigawa, Kano and Katsina states respectively were significantly ($P<0.05$) lower compared with honey samples obtained from Zamfara and source. It was observed that the commercial honey samples had a lower phenolic content when compared with control samples obtained directly from the known beekeepers. These differences might be attributed to the effect of adulteration which removes most of the phenolic content in honey samples. In the present study, the phenolic acid results were lower than those found in Nigerian honeys: 60.47-73.41 mg GAE/g (Ukom *et al.*, 2019) but similar to the results obtained from Vietnamese honey (890-1110 mg GAE/kg), and Bangladesh honeys (470-980 mgGAE/kg) reported by Pham-Nhut *et al.* (2022) and Alzaharani *et al.* (2012) respectively, as well as from Romanian honey (230-1250 mg GAE/kg) reported by Islam *et al.* (2012). Wabaidur *et al.* (2020) reported that the content of phenolic acids in Yemeni honey ranged at level of 10.74-86.80 mg GAE/100g. Chaturvedi *et al.* (2014) reported the phenolic content of processed honey samples from central India in the range of 80.5 to 147.5 mg GAE/kg while the unprocessed samples showed the highest content of 765.3 and 814 mg GAE/kg. Previous studies have mentioned a high gallic acid contents in many honeys. Gallic acid was also well documented phenolic acid responsible for the antioxidant activity of honeys (Cheung *et al.*, 2019). General observation can be made that dark honeys were characterized by considerably higher phenolic content than light coloured honeys.

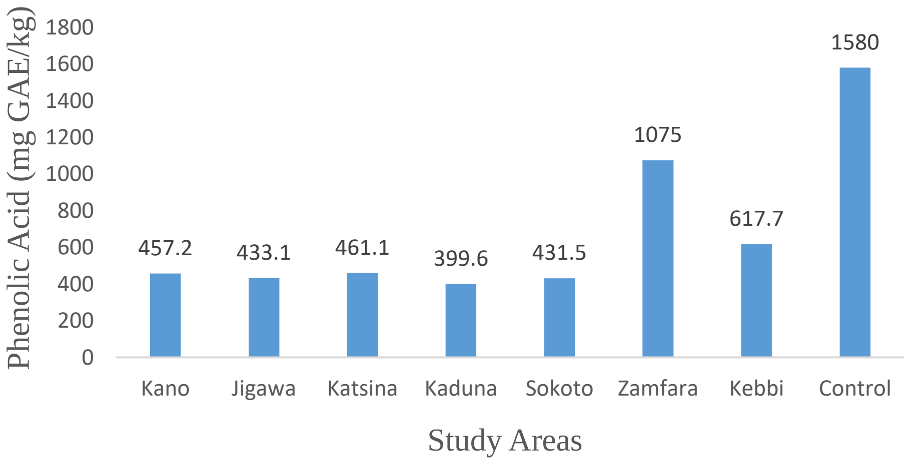


Fig 11: Variation of Mean Concentration of Phenolic Acid (mg/kg) in Honey Samples Analyzed

Table 1: Correlation Coefficient between Flavonoid, Phenolic Acid and Heavy Metals Concentration observed in the Honey Samples.

Heavy Metals	Flavonoid	Phenolic Acid
Zn	-0.09	-0.110
Cd	-0.193*	-0.096
Fe	-0.132*	-0.093
Cu	-0.039	-0.073
Ni	-0.078	-0.022
Pb	-0.058	0.068

NB: Correlation coefficient values with asterisk are significant at P<0.05

Pearson product moment correlation analysis between flavonoid, phenolic acid and heavy metals (Table:1) in the honey samples revealed a significant negative correlation between flavonoid content, Cd and Fe, while most of the heavy metals analyzed (Zn, Cu, Ni and Pb) are non-significant. Phenolic acid content showed a non-significant negative correlation with (Zn, Cd, Fe, Cu and Ni) while a non-significant positive correlation was observed with Pb.

CONCLUSION

The results of this study revealed that most of the commercial honey are contaminated with some heavy metals which may eventually lead to adverse health risks when consumed and losing consumer's trust. Laboratory tests showed that most of the honey samples (about 80%) from open markets are adulterated to some degree and might be through honey harvesting, processing and storage. The study revealed that honey obtained directly from the farms are fairly free from heavy metals contamination. The results obtained from Zamfara and Kebbi states were almost in agreement with standard values or limits and therefore are assumed to be free of adulteration. However, samples obtained from Katsina, Sokoto, Kano, Jigawa and Kaduna were suspected to have undergone some form of adulteration when compared with samples obtained directly from credible beekeepers and standard acceptable limits set by World Health Organization WHO (2015) and NAFDAC (2019).

REFERENCES

Abu-Bakar, M. F., Sanusi, S. B., Abu-Bakar, F. I., Cong, O. J. and Mian, Z. (2017). Physicochemical and Antioxidant Potential of Raw Unprocessed Honey from Malaysian Stingless Bees. *Pakistan Journal of Nutrition,* 16(11): 888-894

Agbajor, G. K. and Otache M. A. (2020). Investigation of Some Physical Properties of Some Nigerian Farm and Local Market Honey Samples. *Nigerian Journal of Science & Environment,* 18(2): 102 – 107

Aghamirlou, H. M., Khadem, M., Rahmani, A., Sadeghian, M., Mahvi, A.H., Akbarzadeh, A. and Nazmara, S. (2015). Heavy Metals Determination in Honey Samples Using Inductively Coupled Plasma-Optical Emission Spectrometry. *Journal of Environmental Health Science and Engineering,* 13(39). DOI: 10.1186/s40201-015-0189-8

Aljedani, D. M. (2022). Determination of Some Heavy Metals and Elements in Honeybee and Honey Samples from Saudi Arabia. *Entomology and Applied Science Letters,* 4(3): 1 – 11

Altun, S. K., Dinc, H., Paskoy, N., Fuson, K. T. and Mehmet, S. (2017). Analyses of Mineral Content and Heavy Metals of Honey Samples from South and East Region of Turkey Using ICP-MS. *International Journal of Analytical Chemistry,* 17 (3): 81-87

Altunatmaz, S. S., Tarhan, D., Aksu, F., Ozsobaci, N. P., O., M. E. and Barutcu, U. B. (2018). Level of Chromium, Copper, Iron, Magnesium, Manganese, Selenium, Zinc, Cadmium, Lead and Aluminium of Honey Varieties produced in Turkey. *Food Science and Technology,* 39(2): 392-397

Alzaharani, H. A., Boukraa, L., Bellik, Y., Abdellah, F., Bakhotmah, B. A., Kolayli, S. and Sahin, H. (2012). Evaluation of the Antioxidant Activity of Three Varieties of Honey from Different Botanical and Geographical Origins. *Global Journal of Health Science,* 4(6): 191-196

Bakchiche, B., Temizer,L, K., Guder, A., Celemili, G., Yegin, S. C., Bardaweel, S. K. and Ghareeb, M. A. (2020). Chemical Composition and Biological Activities of Honey Bee Products from Algeria. Journal of *Applied Biotechnology Reports,* 7(2): 93-103

Batista, B. L., Silva, L., Rocha, B. A., Rodrigues, J., Beretta, A., Bonates, T., Gomez, V. S. D., Barbosa, R. M. and Barbosa, F. (2012). Multi-Element Determination in Brazilian Honey Samples by Inductively

Coupled Plasma Mass Spectrometry and Estimation of Geographic Origin with Data Mining Techniques. *Food Research International*, 49(1): 209 - 215

Beshaw, T., Demssie, K. and Leka, I (2022). Levels and Health Assessmentof Trace Metals in Honey from Different Districts of Bench Sheko Zone, Southwest Ethiopia. *Heliyon*, 8(9): 9-17

Chafik, B. M and Adnene, B. I. (2022). Determination of Heavy Metals in Honey Samples from Different Regions of the Northeast of Algeria: According to an Urban Gradient. *Pollution,* 8(3): 820 – 829

Chaturvedi, V., Vaishnaw, M., Bhatt, R. and Verma, P. (2014). Variation in Antioxidative Potential of Processed and Unprocessed Honey Samples from Central India. A Plausible Role of Quinolin and Gallic Acid as Antioxidants. *Current Trends in Biotechnology and Pharmacy.* 8(3): 264-275

Chua, L. S., Norul, L. A. R., Nur, A. A., and Tjih, T. T. (2013). Antioxidant Activity of Three Honey Samples in relation with Their Biochemical Components. *Journal of Analytical Methods in Chemistry.* Article ID 313798. http://dx.doi.org/10.1155/2013/313798. Retrieved on 11th, April, 2020

Chukwujindu, M. A., Grace, E. O., Godswill. O. T., Grace, O. and Francisco, I. B. (2015). Concentration of Selected Metals in Honey Consumed in Nigeria. *International Journal of Environmental Studies,* 72(4): 713 – 722

Chuleeporn, S., Anothai, T. and Bundit, T. (2018). Micro Mineral Contents in Thai Honey. *International Journal of Applied Engineering Research,* 13(6): 4153 – 4156

Costa, A., Veca, M., Barberis, M., Tosti, A., Notaro, G., Nava, S., Lazzari, M., Agazzi, A. and Tangorra, F. M. (2019). Heavy Metals on Honeybees Indicate Their Concentration in the Atmosphere. A Proof of Concept. *Italian Journal of Animal Science*, 18(1): 309 – 315

El-Sohaimy, S. A., Masry, S. H. D. and Shehata, M. G. (2015). Physicochemical Characteristics of Honey from Different Origins. *Annals of Agricultural Science,* 60(2): 279 – 287.

Ernest, E., Onyeka, O., Ozuah, A. C., and Benard, N. (2018). Comparative Assessment of the Heavy and Trace Metal Levels in Honey Produced within Nsukka and Enugu Metropolis. *Food and Public Hea*lth, 8(2): 42 – 46.

Esubalew, A., Ariaya, H., Gebremariam, B. W. and Ayenew, A. (2020). Determination of Some Heavy Metals in Honey from Different Regions of Ethiopia. *Cogent Food and Agriculture*, 6 (1), DOI: 10. 1080/23311932.2020.1764182

Eteraf-Oskouei, T. and Najafi, M. (2012). Traditional and Modern Uses of Natural Honey in Human Diseases: A Review, *Iranian Journal of Basic Medical Sciences,* 16: 731 – 742

Gul, A. and Pehlivan, T. (2018). Antioxidant Activity of Some Monofloral Honey Types Produced Across Turkey. *Saudi Journal of Biological Sciences*, 25(6): 1056-1065

Haouam, L., Tahar, A., Dailly, H., Lahrichi, A., Chaqroune, A. and Abdennour, C. (2016). Physicochemical Properties and Major Elements Contents of Algerian Honeys from Semi- Arid Regions. Emirates *Journal of Food and Agriculture,* 28 (2): 107-115

Idoko, J. O., Ijege, K. O., Haruna, B. S., Tifwa, P. A. and Musa, W. O. (2018). Evaluation of Heavy Metals in Honey from Brinin Gwari (Nigeria). *Journal of Chemical Society of Nigeria,* 43 (1): 99–103

Islam, A., Khalil, I., Islam, N., Moniruzzaman, M., Mottalib, A., Sulaiman, S. A. and Gan, S. H. (2012). Physicochemical and Antioxidant Properties of Bangladesh Honeys Stored for More than One Year. *BMC Complementary and Alternative Medicine,* 12(1): 177-186

Ita, B. N. (2011). Antioxidant Activity of Honey Samples from Southern Rainforest and Northern Savannah Ecosystems in Nigeria. *International Journal of Pharmaceutical Sciences and Research,* 2(8): 2115-2120

Iwegbue, C. M. A., Obu-Iyeke, G. E., Tesi, G. O., Obi G. and Bassey, F. I. (2015). Concentrations of Selected Metals in Honey Consumed in Nigeria. *International Journal of Environmental Studies,* 72(4): 713–722

Kaya, B. and Yildirim, A. (2021). Determination of the Antioxidant, Antimicrobial and Anticancer Properties of Honey Phenolic Extract of Five Different Regions of Bingol Province. *Journal of Food Science and Technology,* 58: 2420-2430.

Lewoyehu, M., Amare, M. and Yildiz, F. (2019). Comparative Evaluation of Analytical Methods for Determining the Antioxidant Activities of Honey: A Review. *Cogent Food and Agriculture*, 5:(1), 1685059. DOI: 10.1080/23311932.2019.1685059

Mahmoudi, R., Mardani, K., and Rahimi, B. (2015). Analysis of Heavy Metals in Honey from North-Western Regions of Iran. *Journal of Chemical Health Risks,* 5(4): 251 – 256.

Majewska, E., Druzynska, B. and Wolosiak, R. (2019). Determination of the Botanical Origin of Honeybee Honeys Based on the Analysis of their Selected Physicochemical Parameters Coupled with Chemometric Assays. *Food Science and Biotechnology*, 28: 1307 – 1314.

Maria, de T. C. L., Maia, de S. M., Emanuel, de C. C., M., Islay, L. M., Daniel, B. C. and Maciel, de M. O. S. (2013). Physicochemical Properties and Mineral and Protein Content of Honey Samples from Ceara State, Northeastern Brazil. *Food Science and Technology, (Campinas)*, 33(1): 38 – 46.

Marschner, P. (2012). Marschner's Mineral Nutrition of Higher Plants. 3rd Ed. London, UK: Academic Press Elsevier. Retrieved on 13th January, 2020.

Melaku, M. and Tefera, W. (2022). Physicochemical Properties, Mineral and Heavy Metal Contents of Honey in Eastern Amhara Region, Ethiopia. *Journal of Food Composition and Analysis.* 114, 104829, ISSN 0889 – 1575, https://doi.org/10.1016/j.jfca.2022.104829 . Retrieved on 11th October, 2022.

Moniruzzaman, M., Chowdhury, M. A. Z., Abdur Rahman, M., Sulaiman, S. A., and Gan, S. H. (2014). Determination of Mineral, Trace Element, and Pesticide Levels in Honey Samples Originating from Different Regions of Malaysia Compared to Manuka Honey. *Biomed Research International*, http://dx.doi.org/10:1155/2014/359890. Retrieved on 16th July 2019.

Moniruzzaman, M., Sulaiman, S. A., Khalil, M. I., and Gan, S. H. (2013). Evaluation of Physicochemical and Antioxidant Properties of Sourwood and Other Malaysian Honeys: A Comparison with Manuka Honey. *Chemistry Central Journal*, 7: 138-149.

Mutlu, C., Erbas, M., & Tontul, A. S. (2017). Some Characteristics of Honey and Other Bee Products and their Effects on Human Health. *Academic Food*, 15(1): 75-83.

National Agency for Food and Drug Administration and Control, NAFDAC (2019). National Regulatory Authority. Food Safety Prepare for the Unexpected. Google Scholar. Retrieved on 2nd March, 2020.

Ndife, J., Kida, F. and Makarfi, T. (2014). Quality Assessment of Nigerian Honey Sourced from Different Floral Locations. *Journal of Food and Nutrition Sciences.* 2(4): 162 – 167.

Njokuocha, R. C., Dim, K. I., Onyejekwe, O. K., and Nwokorie, V. U. (2019). Determination of the Concentration of Some Mineral Elements and Pollen Spectra of Apis mellifera L. Honeys from Different Locations in Nigeria. *Animal Research International*, 16(1): 3186–3197.

Okeola, F. O., Oluade, O. and Liad, M. T. (2020). Stingless Bee Honey as Bio-Indicator of Heavy Metals Pollution in and around the University of Ilorin Environ, Kwara State, Nigeria. *Journal of Applied Science and Environmental Management*, 24(5): 773 – 778.

Pham-Nhut, T., Nguyen, T. V., Le-Truong, D., Diep, L. M. N., Nguyen, K. N., Nhu, T. H., Le-Hung, T. and Nguyen, Q. V. (2022). Phenolic Profiles, Antioxidant, Antibacterial Activities and Nutritional Value of Vietnamese Honey from Different Botanical and Geographical Sources. *AgriEngineering*, 4(4): 1116-1138.

Ramanauskiene, K., Stelmakiene, A., Briedis, V., Ivanauskas, L. and Jakstas, V. (2012). The Quantitative Analysis of Biologically Active Compounds in Lithuanian Honey. *Food Chemistry*, 132(3): 1544-1548.

Salihaj, M. and Bani, A. (2017). The Nickel Content in Honey Derived from Serpentine and Non- Serpentine Areas of Kosovo. *Albanian Journal of Agricultural Science*, Special edition (Open Access), 557 – 563.

Sime, D., Atlabachew, M., Abshiro, M. R. and Zewde, T. (2015). Total Phenols and Antioxidant Activities of Natural Honeys and Propolis Collected from Different Geographical Regions of Ethiopia. *Bulletin of Chemical Society of Ethiopia*, 29(2): 163- 172.

Singh, C., Shubharani, R. and Sivaram, V. (2014). Assessment of Heavy Metals in Honey by Atomic Absorption Spectrometer. *World Journal of Pharmacy and Pharmaceutical Sciences,* 3(8): 509 – 515.

Stecka, H., Jedryczko, D., Welna, M. and Pohl, P. (2014). Determination of Traces of Copper and Zinc in Honeys by the Solid Phase Extraction Pre-Concentration Followed by the Flame Atomic Absorption Spectrometry Detection. *Environmental Monitoring and Assessment* 186(10): 6145-6155.

Tibebe, D., Hussen, M., Mulugeta, M., Yenealem, D., Moges, Z., Gedefaw, M. and Kassa, Y. (2022). Assessment of selected Heavy Metals in Honey Samples Using Atomic Absorption Spectroscopy (AAS), Ethiopia. *Research Square.* DOI: https://doi.org/10.21203/rs.3.rs.16824495/v1.

Toma, I, Jefferson, J. N., Clifford, L. B. and Mofio, B. M. (2020). Assessment of Heavy Metals and Pesticide Residues in Honey Samples collected from Selected Villages in Five Local Government Areas of Adamawa State, Nigeria. *International Journal of Scientific and Research Publications,* 10(9): 383 – 392

Tutun, H., Kahraman, H. A., Aluc, Y., Avci, T. and Ekici, H. (2019). Investigation of some Metals in Honey Samples from West Mediterranean Region of Turkey. *Veterinary Research Forum;* 10(3): 181 – 186

Ukom, A. N., Okereke, I. O. and Ugwuona, F. U. (2019). Physicochemical Properties and Antioxidant Activities of Honey from Southern Nigeria. *Journal of Chemical Society of Nigeria.* 44(6): 1044-1055

Wabaidur, S., Obbed, M. S., Alothman, Z. A., Alfaris, N. A., Badjah-Hadj-Ahmed, A. Y., Siddiqui, M. R., Altamimi,J. Z. and Aldayel, T. S. (2020). Total Phenolic Acids and Flavonoid Contents Determination in Yemeni Honey of Various Floral Sources: Folin-Ciocalteu and Spectrophotometric Approach. *Food Science and Technology.* In Press. http://dx.doi.org/10.1590/fst.33119

WHO (2015). World Health Organization Expert Committee on Food Additives. Summary and Conclusion in; 74th meeting, Rome, 18 - 23