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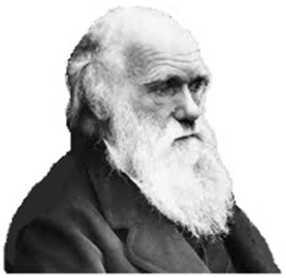

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Pollen analysis and heavy metals detection in honey samples from southern Nigeria

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ABSTRACT

Pollen analytical study of two locally produced honey samples, collected from Akwa Ibom and Cross River State, Southern Nigeria, were carried out in order to ascertain the preferentially foraged plants by honey bees, as well as the concentration of heavy metals. Samples were subjected to standard laboratory procedures using an acetolysis mixture (pollen analysis) and atomic absorption spectrometry (heavy metals). The results from the analysis revealed 32 taxa belonging to 17 botanical families. The number of pollen grains in the honey samples varied significantly (between 4,887 - 12,755 pollen grains), indicating their richness in pollen grains. 12 elements – Ni, Cu, Zn, Se, Br, K, Ca, Ti, Cr, Mn, Rb, and Fe were detected. Potassium had the highest concentration, followed by iron, calcium, titanium, zinc, copper, chromium, nickel, among others. What is more, in this study, some important honey plants: *Spondias mombin, Daniella oliveri, Manihot esculenta, Treculia africana, Syzigium guineensis*, *Diospyros mespiliformis*, *Parkia biglobosa, Terminalia superba*, *Senna hirsuta,* among others, were found to be predominant and have been identified to be characteristic of the vegetation typical of Southern Nigeria. With regard to honey, this study gives an indication of the geographical and botanical origins, as well as types, source, and degree of contamination, and also an overall measure of honey purity. Such information, when displayed, can help consumers make informed decision when purchasing honey and will also help beekeepers to avoid possible contamination. Moreover, it will assist regulatory agencies in taking proper measures for environmental and consumer protection, since the concentration of heavy metals in honey is influenced by environmental pollution.

***Keywords*:** Honey, Pollen, Heavy metals, Quality, Akwa-ibom, Cross River, *Spondias mombin, Daniella oliveri, Manihot esculenta, Treculia africana, Syzigium guineensis, Diospyros mespiliformis, Parkia biglobosa, Terminalia superba, Senna hirsute*

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1. INTRODUCTION

Honey is a sweet, viscous food substance produced by bees from the nectar of flowers. Bees produce honey from the sugary secretions of plants by regurgitation, enzymatic activity, and water evaporation. There are four natural resources required by honeybees for survival: water, resin, nectar, and pollen (Seedley, 1985). Water is used to cool hive and to dilute honey fed to larvae. Resin is utilized to reinforce the hive, seal off decaying wood, and plug up holes (Bibi *et al.*, 2008). Nectar is the major source of carbohydrates from which honeybees obtain their energy. It is collected by foraging worker bees and is carried back to hive in their honey stomachs. Nectar is usually transferred to hive workers for processing into honey and it can be fed directly to the brood or to adults (Winston, 1987). During nectar gathering, a honeybee consumes 0.5 mg of ripe honey per kilometer of flight. Feeding a bee larva from egg to maturity requires about 142 mg of honey (Winston, 1987). Honey consists essentially of carbohydrates, amino acids, minerals, vitamins, enzymes, pollen and pigments (Schramm *et al*., 2003). Emphasis has always been on the nectar of foraged plants (Jantakee and Tragoolpua, 2015) for their bio active compounds. Nevertheless, pollen is the bee's major source of protein, fat, minerals, and vitamins, while nectar is the major source of carbohydrates from which honeybees obtain their energy (Adekanmbi and Ogundipe, 2009). When the nectar has been converted into honey in the hive, some of the pollen remains in the honey (Engel and Grimaldi, 2005). This is borne out of the fact that not only is honey useful as a food supplement; but it is now increasingly being used in the treatment of various diseases (Molan, 2001). These healing properties of honey are as a result of the integration of pollen and nectar containing bio active ingredients from medicinal plants that the bees foraged on. Its nutritional, medicinal and sensory properties have attracted thousands of consumers (Carlos *et al*., 2009). Thereby, creating employment and room for the adulteration of honey through the demand and supply gap in Nigeria.

Pests and diseases have been reported to cause about 15% decline in honey bee colony establishment, as well as the regular absconding and aggressiveness of the honeybees, thereby, leading to large-scale sales of adulterated products in our markets. Presently, Nigerian honey is not sold under any standard control or characterization that is significantly different from the claims of the beekeepers (Aina, 2016). This is not the case with European Economic Union that has had strict labeling regulations for honey products since 1974. Because of the trade agreements, import tariffs and legal trade restrictions, most of the leading honey-producing nations of the world require accurate labeling of honey before it can be sold.

Analytic method, such as pollen analysis, is of great importance for quality control of honey. Since that, they have been reported to contain numerous pollen grains and honeydew elements that provide a good fingerprint of the environment where the honey is produced. This analysis can be useful to determine the geographical and botanical origin. Pollen is an essential tool in the analysis of honey as it indicates the major and minor plant taxa utilized by honeybees. Also, they serve as useful indicators of the local and regional plant species visited by the honeybees as nectar sources. Furthermore, pollen analysis provides some important information about honey extraction and filtration, fermentation (Russmann, 1998), some kinds of adulteration (Kerkvliet *et al*., 1995) and hygienic aspects such as contamination with mineral dust, soot, or starch grains (Louveaux *et al*., 1978), studying allergies, climatic change or solving forensic riddles (Erdtman, 1969). In order to have a beneficial effect, honey must be free from every contaminating agent, any contaminants, such as heavy metals present in honey

above the permissible limits by pollution standards, as being threats to human health. The current international honey market trend, regarding quality, is more demanding. Therefore, it is necessary to promote all feasible activities in order to produce residue free honey (McKee, 2003). As a result of industrial development, the production and emission of heavy metals have increased. The dispersion of metals on plants parts may directly end up in the food chain.

The order in which pollen grains are domicile may be polluted, may result in the polluted honey. Fakhimzadeh and Lodenius (2000) are of the view that to produce half a kilogram of honey, bees have to visit 3-4 million of flowers and collect 75,000 nectar loads to their colony. Since honey is a nutritional resource that depends on both, biotic and abiotic factors around the beehives, the presence of heavy metals could be related to its geographical and botanical origin.

Different studies have indicated that almost all macro-minerals were commonly found in honeys from all countries, with the exception of Cl, which was only detected in honey samples from Spain (Gonzalez Paramas *et al*., 2000). Hence, it is important to take into account the type of equipment used to produce and store honey after harvesting, as they could act as possible sources of honey contamination with heavy metals. Contact with stainless steel surfaces during harvesting and processing of honey for the market, can generate a high Chromium content, due to the corrosive effect of honey acidity. Likewise, storing honey in galvanized containers can be a source of Zinc contamination (Bogdanov *et al*., 2003).

This study was carried out to identify and measure the concentration of heavy metals present in honey samples from Cross River and Akwa Ibom State, Southern, Nigeria. Plant species were also identified from pollen sculpturing found in honey samples, and to point out adulteration in honey from possible different sources, which will enhance the quality and acceptability in the local/international market.

2. MATERIALS AND METHODS

2. 1. Study areas

Locally produced honey samples were collected from two states in Southern, Nigeria; they are Obudu in Cross River which lies within latitude 40°41'South and 60°30' North and between longitude 8° and 9°00' East of the equator, Itu Local Government area of Akwa Ibom State. Itu is located at 5°10'0"N and 7°59'0"E and bounded in the North by Odukpani in Cross river and Arochukwu in Abia state, in the West by Ibiono Ibom and Ikono Local Government Areas, while in the South by Uyo and Uruan Local Government areas (**Figure 1**), respectively. The areas receive 1,718 to 2,190 mm of rainfall annually which lasts from October to February, while the dry season lasts from November through February.

The mean annual temperature and relative humidity are generally high for most parts of the year, showing drastic drops, however, during the harmattan period (December - January) when there is a considerable cloud cover in the atmosphere. Also, the mean annual temperature varied from 74 to 79%. The climate of the study areas could therefore be described as sub-humid. The climax vegetation consisted of scattered low-growing, and drought resistant trees which include, *Butyrospermum parkii, Alchornia cordifolia, Gmelina arborea Aspilia africana Elaeis guineensis, Chlorophora excelsa, Dacryodes edulis* and *Pennisetum purpureum* which were dominant. The vegetation of the study areas could therefore be described as rain forest/savanna mosaic.

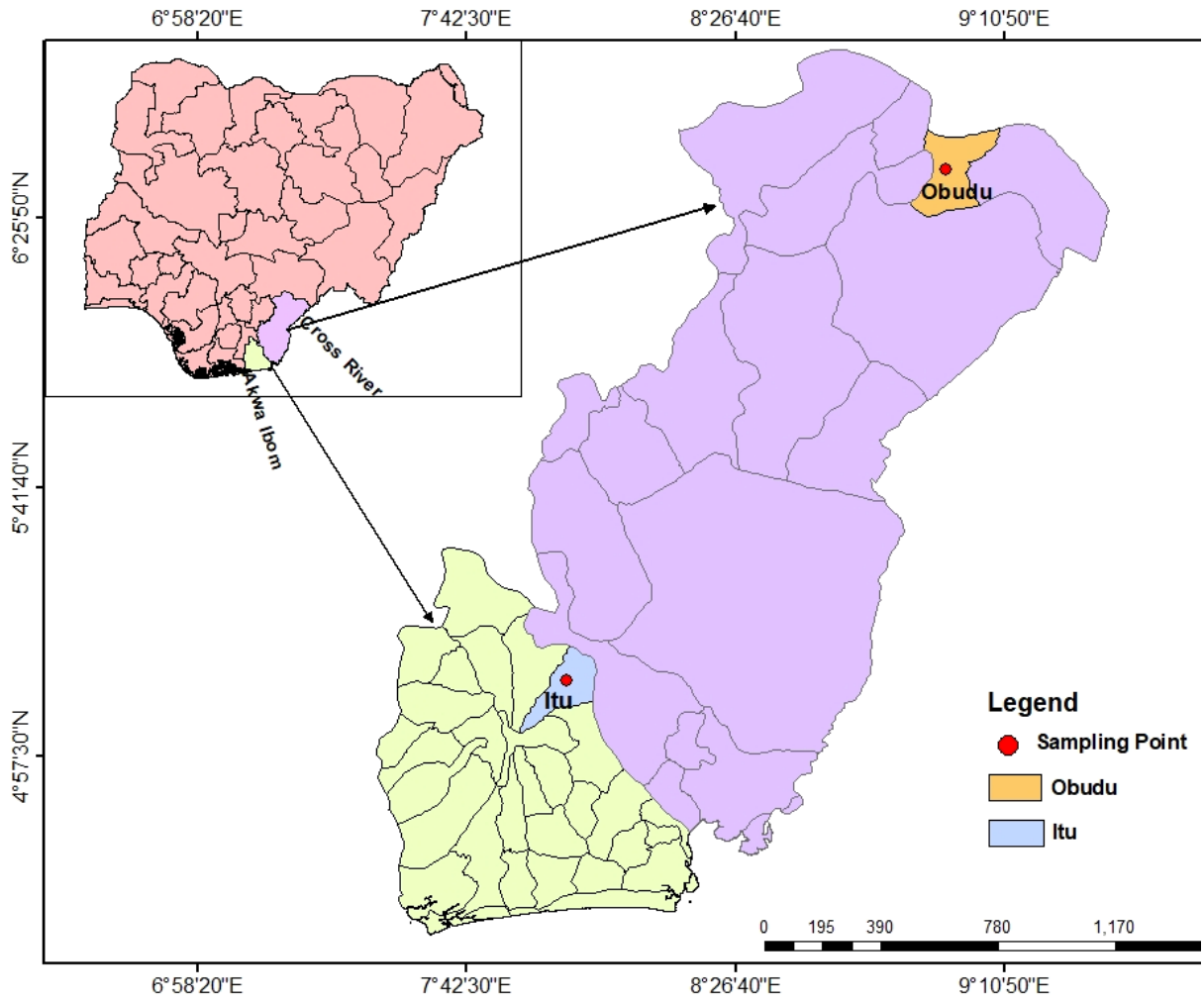

Fig. 1. Map showing the sample locations at Itu in Akwa Ibom and Obudu in Cross River State, South-South, Nigeria

2. 2. Sample preparation

Five grams of honey samples from each sub-sample were weighed and poured into test-tubes and acetolyzed according to Erdtman's (1969) method. Glycerine (0.5 mL) was added to each sample and the storage bottles were labeled, respectively. Micropipette was used to transfer 0.1 mL of the residue on glass slides and cover-slipped with sealant. Each slide was observed under Olympus 2.0 light microscope and the recovered pollens were counted and recorded.

The view count method was used in the palynological analysis, of which 20 representative focal points were picked on each slide and studied. Identification was made using published floras and West African atlases, such as Sowunmi (1995), Agwu and Akanbi (1985), Gosling *et al*., (2013), as well as the reference slide collection of the Laboratory of Palynology/ Palaeobotany, Department of Botany, University of Lagos, Akoka, Lagos. Photomicrographs of some important pollens were taken with a Motic camera 2.0.

The pollen was identified and recorded using the view count method of (Louveaux *et al*., 1978). The classification method of (Vergeron, 1964) was adopted for expressing the frequencies of pollen grains in honey. This includes "very frequent" for grains constituting more than (45%), ''frequent'' (16-45%), "sporadic'' for grain constituting less than 3% or rare (3-15%).

2. 3. Proximate and mineral analysis

Moisture, ash, crude protein, fat contents and dietary fiber were evaluated by the methods described in Association of Analytical Chemist (1999). Moisture content was determined by drying 4 g of each sample in an oven at 100 °C for 3 hours to constant weight in hot air-oven. The ash content was determined by the incineration of a 4 g of each sample in a porcelain vessel at 105 °C for 3 hours in hot air-oven.

The dried samples were ignited in an ash in a furnace at 600 °C for 6 hours to constant weight, until the ash turned white. For the trace mineral elements, the ash of each honey sample obtained was digested by the addition of 5 mL of 2 M HCl in a crucible and heated to dryness on a heating mantle. Five microliters of 2 M HCl were added afterwards, heated to boil and filtered through a Whatman No. 1 filter paper into a 100-mL volumetric flask. The filtrate was made up with distilled water stopper and made ready for reading. These diluents were aspirated into the Buck 211 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at its respective wavelength with their respective cathode lamps, using appropriate fuel and oxidant combination (AOAC, 1999). The pH/conductivity of honey samples were measured using a pH meter (Hanna Instruments). The colour of the samples was determined using the Lovibond comparator, while the refractive index measurements were done with an Abbe refractometer.

3. RESULTS

The pollen analysis of honey samples from Itu in Akwa Ibom state and Obudu in Cross River state revealed the abundance of pollen grains which reflected the habit, as well as the conservation status of these areas (**Table 1**). A total of 32 pollen types was recovered, belonging to 17 families, while 137 pollen grains were recorded as pollen indeterminate. In Itu honey samples two predominant families were frequently encountered during the analysis and contributed the highest number of the pollen recovered totaling 2,667.

These families are Fabaceae 1,159 (23.7%) from eight taxa and Euphorbiaceae 662 (13.5%) from four taxa (Table 1). Pollen spectra of honeys revealed a variety of not only nectariferous but also nectarless sources available to bees and included taxa whose presence indicated their origin. Such taxa include *Spondias mombin, Daniella oliveri, Manihot esculenta, Treculia africana, Syzigium guineensis*, *Diospyros mespiliformis*, *Parkia biglobosa*, *Terminalia superba*, *Senna hirsuta* among others were predominant.

The pollen composition of the honeys studied revealed important information on the flora of that region. The heavy metals analysis indicates that potassium has the highest concentration, followed by iron, calcium, titanium, zinc, copper, chromium, and nickel (**Tables 2**, **3**). The lowest iron concentration was found as 121 ppm in honey sample from Cross River and the highest concentration was found as 263 ppm in honey sample from Akwa Ibom.

Table 1. Palynomorphs recovered from Itu and Obudu honey samples

		AkwaIbom State (Itu) Honey			Cross-River State (Obudu) Honey		
S.N	**Plant Taxa**	**Pollen count**	**Relative abundant (%)**	**Frequency class**	**Pollen count**	**Relative abundant (%)**	**Frequency class**
1.	*Elaeis guineensis* Jacq	211	4.3	Rare	2531	19.8	Frequent
2.	*Chromolaena odorata*	87	1.7	Sporadic	32	0.2	Sporadic
3.	*Albizzia zygia* (DC.) Macbr	34	0.6	Sporadic	129	1.0	Sporadic
4.	*Mangifera indica* L	22	0.4	Sporadic	42	0.3	Sporadic
5.	*Parinari curatellifolia* Planch ex Benth	62	1.2	Sporadic	54	0.4	Sporadic
6.	*Berlinia grandiflora* (Vahl) Hutch. & Dalziel	11	0.2	Sporadic	234	1.8	Sporadic
7.	*Terminalia superba* Engl. & Diels	58	1.2	Sporadic	606	4.7	Rare
8.	*Isoberlinia doka* Craib & Stapf	12	0.2	Sporadic	-	-	-
9.	*Senna hirsuta* L	855	17.5	Frequent	328	2.5	Sporadic
10.	*Rauvolfia vomitoria* Afzel	96	1.9	Sporadic	49	0.3	Sporadic
11.	*Nauclea latifolia* Smith	44	0.9	Sporadic	91	0.7	Sporadic
12.	*Bombas buonopozenese* P. Beauv	1812	37.0	Frequent	1201	9.4	Rare
13.	Pollen indeterminate	86	1.7	Sporadic	51	0.3	Sporadic
14.	*Azadirachta indica* Juss.	14	0.2	Sporadic	-	-	-
15.	*Allophyllus africanus* P. Beauv.	31	0.6	Sporadic	24	0.1	Sporadic
16.	*Parkia biglobosa* (Jacq) R. ex Don H.C	48	0.9	Sporadic	885	6.9	Rare
17.	*Lannea acida* A. Rich	11	0.2	Sporadic	-	-	-
18.	*Luffa cylindrica* M. J. Roem	19	0.3	Sporadic	-	-	-
19.	*Phyllantus muellerianus* (O. Ktz) Exell	27	0.5	Sporadic	11	0.08	Sporadic
20.	*Daniella oliveri* (Rolfe) Hutch& Dalz	82	1.6	Sporadic	1112	8.7	Rare
21.	*Bridelia micrantha* (Hochst.) Baill	91	1.8	Sporadic	56	0.4	Sporadic
22.	*Manihot esculenta* Crantz	441	9.0	Rare	508	3.9	Rare

23.	*Diospyros mespiliformis* Hochist xe ADC	231	4.7	Rare	114	0.8	Sporadic
24.	*Treculia africana* Decne	-	-	-	885	6.9	Rare
25.	*Andropogon gayanus* Kunth var. gayanu	71	1.4	Sporadic	12	0.09	Sporadic
26.	*Dalium guineense* Willd	14	0.2	Sporadic	14	0.10	Sporadic
27.	*Anacardium occidentalis* L	39	0.7	Sporadic	45	0.35	Sporadic
28.	*Ageratum conyzoides* L	67	1.3	Sporadic	109	0.85	Sporadic
29.	*Alchornia cordifolia* Muell, Arg.	103	2.1	Sporadic	1429	11.2	Rare
30.	*Trichillia prieureana* A Juss.	52	1.0	Sporadic	109	0.8	Sporadic
31.	*Spondias mombin* L	-	-	-	987	7.7	Rare
32.	*Entada abyssinica* Steud. Ex A. Rich.	103	2.1	Sporadic	204	1.5	Sporadic
33.	*Syzygium guineensis* Willd. DC.	94	1.9	Sporadic	903	7.0	Rare
	Total	**4,887**			**12,755**		

Table 2. Physicochemical Analysis of Honey samples from Itu and Obudu

S.N	Sample area	pH	Refractive index	Conductivity (μscm^{-1})	Colour (pt.Co)	Moisture content (%)	Ash content (%)
1.	Itu LGA (Akwaibom)	4.5	1.467	0.02	129.50	17.45	0.32
2.	Obudu (Crossriver)	4.4	1.472	0.01	62	16.27	0.41

Table 3. Trace elements analysis of Honey samples from Itu and Obudu

S.N	Sample area (Mg/g)	Se	Zn	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Br	Rb
1.	Itu LGA (Akwaibom)	3.4	32	2800	218	78	4	1	263	3	14	2.8	5.4
2.	Obudu (Crossriver)	2.3	43	2210	255	44	11	3	121	12	26	2.1	5.9

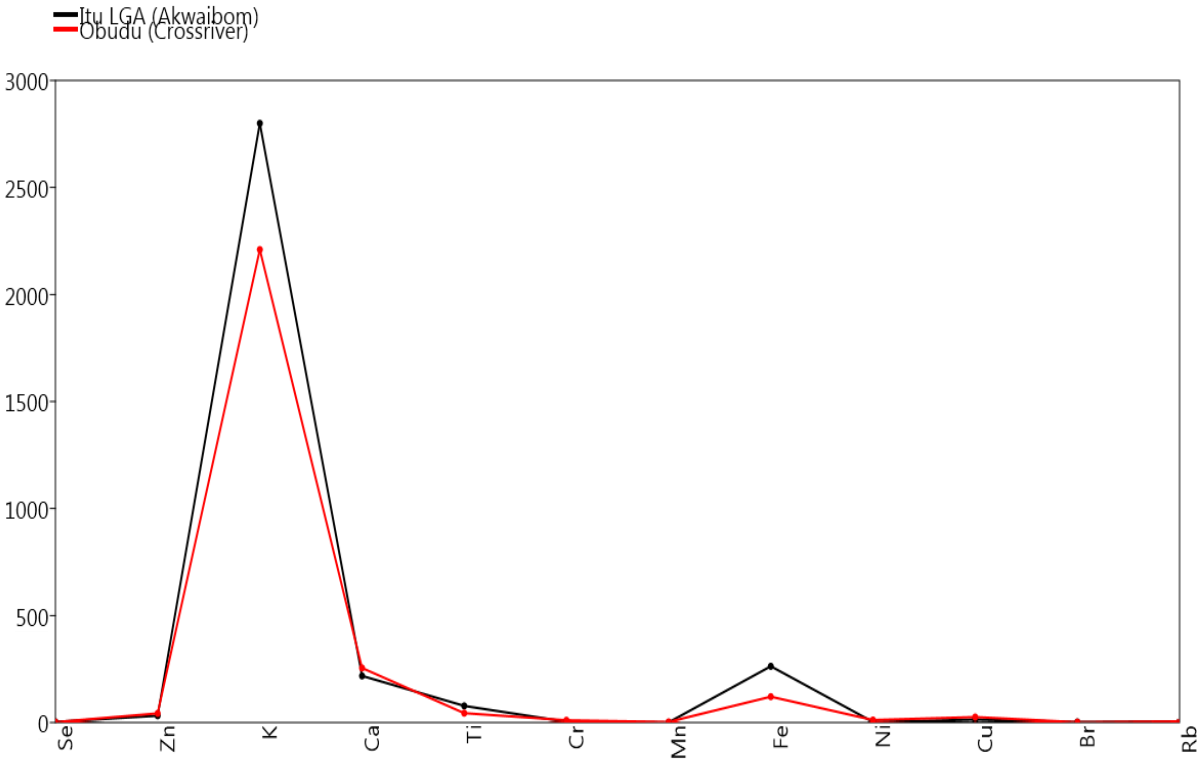

Figure 2. Levels of heavy metals between Honeys sample of Cross River and Akwa Ibom

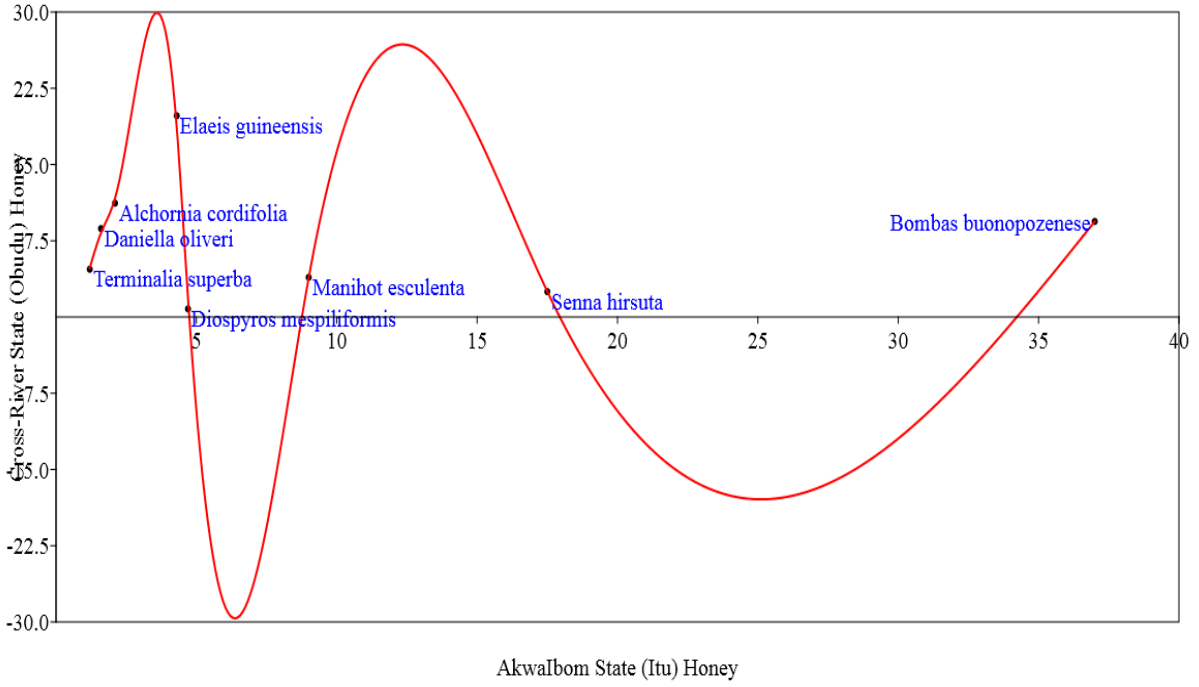

Figure 3. Predominant honey plants identified in two honey samples from Southern Nigeria

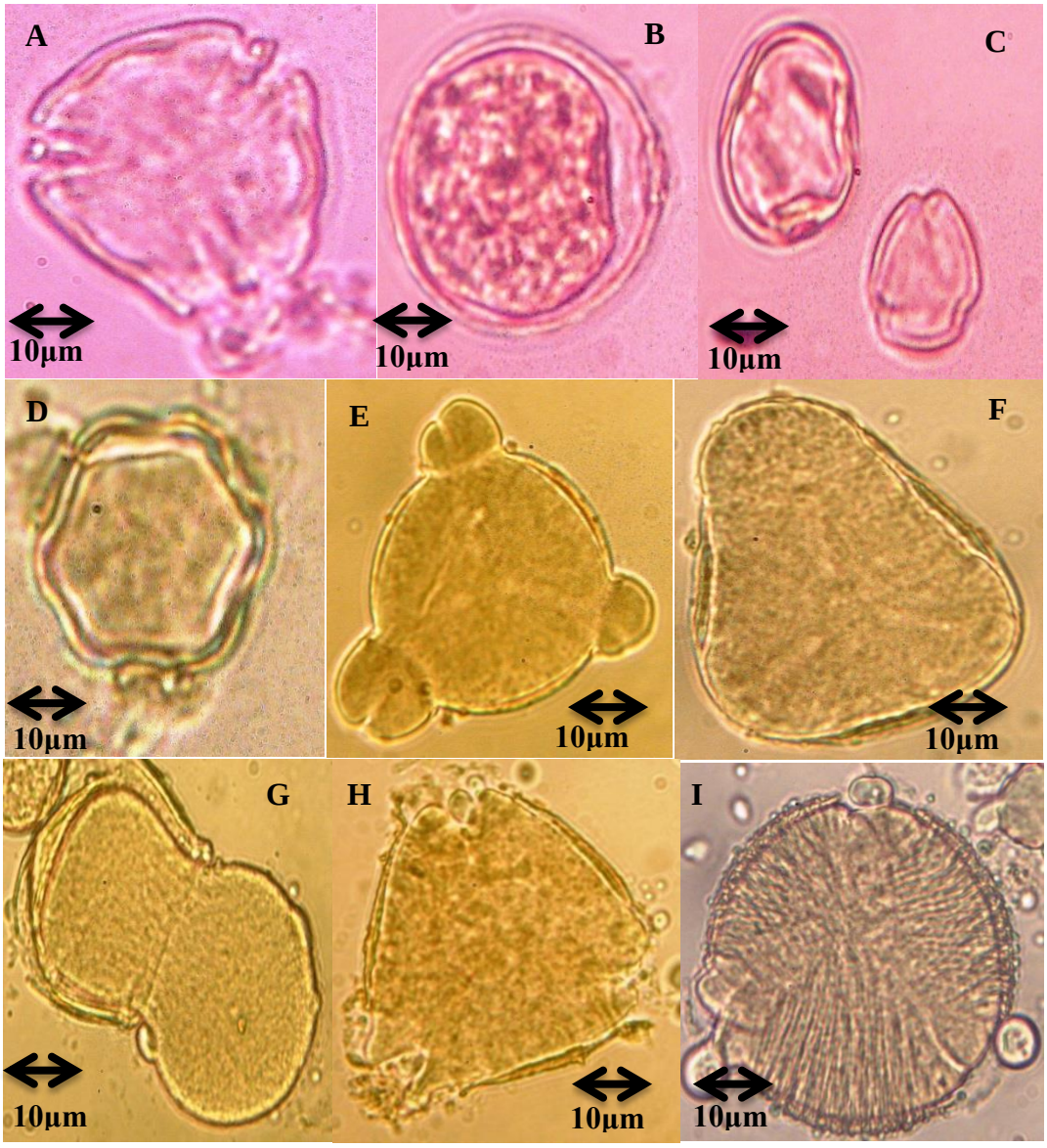

Figure 4. A - *Rauvolfia vomitoria*, B - *Andropogon gayanus*, C - *Nauclea latifolia*, D - *Terminalia superba*, E - *Senna hirsute*, F - *Elaeis guineensis*, G - pollen indeterminate, H - *Parinari curatellifolia*, I - *Isoberlina doka*.

The lowest manganese level was 1 ppm in honey sample from Akwa Ibom, and the highest manganese level was 3 ppm in honey sample from Cross River (**Figures 2**, **3**) . The lowest zinc content was found 32 ppm in Akwa Ibom sample and the highest zinc content 43 ppm was in honey sample from Cross River. The minimum copper concentration observed was 14 ppm in honey sample from Akwa Ibom and maximum 26 ppm in Cross River honey sample. The lowest selenium content, 2.3 ppm was in Cross River honey and the highest selenium content was 3.4 ppm in honey sample from Akwa Ibom. The pH level ranges from (4.4- 4.5), while the refractive index was (1.46-1.47), conductivity (19.2-22.1 $\mu s \cdot cm^{-1}$), colour (62-129 pt.Co), moisture content (16.2-17.4%), and ash content (0.3-0.4%) for both honey samples (**Figure 4**).

4. DISCUSSION

The pollen analyses showed that the honey samples had moderately abundant and diversified pollen grains. Fabaceae family members were traced as being the dominant group in both honey samples. Euphorbiaceae, Anacardiaceae, Malvaceae, Asteraceae, Arecaceae, Ebenacae, among others, followed Fabaceae in sequential form (Table 1). *Elaeis guineensis* pollens were found to be frequent in Cross River honey sample, as a result of their prolific nature, as well as their high vitamin A content which aid reproduction of honeybees. Crane (1983) is of the view that *Elaeis guineensis* pollen is abundant in honey due to the juice of the fermenting fruit which is collected by the bees, thereby making *Elaeis guineensis* their favorite in any area the plant inhabits. In Akwa Ibom honey samples, *Elaeis guineensis* were found to be rare as a result of over exploitation by humans as sources of food and income. Its pollen is used as food by the bees since the plant does not produce nectar (Aina and Owonibi, 2011).

Apart from Arecaceae and Anacardiaceae, all others are wild plants which are exposed to various degrees anthropogenic activities, i.e. deforestation, bush burning, wild fire, among others. These dominant honeybee plants are indication of a Tropical Rainforest vegetation belt/ lowland forest and secondary vegetation, found in Southern Nigeria. *Senna hirsuta* and *Bombas buonopozenese* pollens were equally frequently encountered in these honey samples from Southern Nigeria. Their families were abundant and are good indicator species reflecting the characteristic plants of the derived savanna. The honey type of both samples shows that more than thirty-two honey plants species were identified from the two samples, given these samples a multiflora honey type. All the taxa recovered were mostly sporadic, rare, and frequent, none was very frequent, i.e. (>45%). This implies that honeybees frequently collect a wide variety of pollen types, but they generally concentrate on a few families/species which have been reported by several authors to contain a high concentration of protein for their survival, reproduction, and development of the colony. The lack of pollen protein can retard or prevent ovary development. Furthermore, honeybees are species specific based on their developmental stages, that is there must be a positive correlation between the plant they foraged on and the activities in their bee hives. The poor pollen spectrum recorded in Akwa Ibom honey samples could be a reflection of loss of bio-resources attributed to impact of high level of anthropogenic activities in this zone. Nevertheless, anthropogenic activities are not the only factors responsible for less dense vegetation in these area, but the specific selection and support of different trees and shrubs serving human nutrition because trees and shrubs have been and still are of greatest importance in the human diet. Thereby, resulting into humans and bees conflict, most times humans get attacked by these bees. Potts *et al.* (2010) is of the view that nutritional stress due to the habitat loss has played an important role in honeybee colony collapse, and thereby stressed the important of protecting and enhancing the availability of floral resources by using policies for efficient environmental management and conservation.

The pH values of the honey samples from the two States were not significantly different from each other; the values ranged between 4.4 and 4.5 with an average of 4.4. Leveen *et al.* (1975), is of the view that the acidic pH of honey is desirable because acidification promotes wound healing by causing oxygen release from haemoglobin. In addition, the pH of honey is low enough to prevent the growth of bacteria on wounds. Honey pH can provide a good indication of its botanical origin, and it can also be used for the prediction of honey degradation during storage. Honeys with pH ranging from 3.5 to 4.5 are said to originate from nectar (Amir *et al*., 2010). The refractive index for both honey samples was not significantly different from

each other, as well as the electrical conductivities. The conductivity of honey samples depends on their ash and acid contents, i.e., the higher the ash and acid contents, the higher the conductivity (Bogdanov, 2009b). Indeed, Piazza *et al.* (1991) had demonstrated a linear relationship between the ash content and electrical conductivity in Italian unifloral honeys described by the equation, C=0.14 + 74A, where C denotes the electrical conductivity in milliSiemens per cm and A denotes the ash content in g/100 g.

The conductivities of the honey samples analysed in this study varied between 0.01 and 0.02 with an average value of 0.015. The international norm specified by both, Codex Alimentarius Commission and European Council (EU) established values >0.8 mS/cm of blossom honeys or blends (mixtures) of blossom honeys and >0.8 mS/cm for honeydew honeys. The conductivity data in this study shows that all the samples fall within the range required by the international standard. The results also indicate that both honey samples are of floral botanical origin. Conductivity is a good criterion for determining botanical origin of honey and today it is determined in routine honey quality control instead of the ash content (Bogdanov, 2009a). The colour of Akwa Ibom honey sample recorded 129 mm, while that of Cross River was at 62 mm. According to the USDA (1985), honey samples with Pfund values of less than 8 mm are categorized as “water white,” between 9 and 17 mm as “extra white,” between 18 and 34 mm as “white,” between 35 and 50 mm as “extra light amber,” between 51 and 85 mm as “light amber,” between 86 and 114 mm as "amber,” and greater than 114 mm as “dark amber.” The Akwa Ibom honey samples could be characterized as ‘dark amber’, while that of Cross River as ‘Light amber’. Honey color mainly differs based on its floral origin, industrial processing methods, temperature, and storage duration (Gonzales *et al*., 1999). The moisture contents fell between (16.2-17.4 g), which is still within the range (<21 g) according to codex Alimentarius. Honey having a high water content is more likely to ferment, which will lead to an increased acidity.

In both honey samples, twelve elements (Ti, Cr, Mn, K, Ca, Fe, Ni, Rb, Cu, Zn, Se, and Br) were detected and their concentrations determined. These shows that both samples were quite rich in minerals. Iron, calcium, titanium, zinc, copper, chromium, and nickel, among others, followed potassium in sequential form based on their concentration. The twelve elements comprise two major elements (Potassium and calcium) which should be present at >50 mg/d, seven trace elements (iron, zinc, selenium, copper, manganese, chromium, and nickel) are required in concentrations <50 mg/d in human beings (Belitz *et al*., 2009), and three ultra trace elements (bromine, titanium, and rubidium) are usually less than 1 µg/g and often present at less than 50 mg/g in the dry matter of the diet (Nielsen 1984; Belitz *et al*., 2009). Potassium and calcium conform to the permissible limits of >50 mg/d, in which they recorded 2,800 mg/g, 218 mg/g, and 2210 mg/g, 225 mg/g for Akwa Ibom and Cross River honey samples, respectively. The presence of potassium needs to be in balance with the circulatory system, like Na, and potassium also assists in nerve functions, heart activity and muscle contraction (Lambert *et al*., 2008). Cacium is an essential mineral because of its important contribution to several biological functions in the cardiac, nervous, and musculoskeletal systems, including contributing to the formation of bone and teeth. Furthermore, Calcium is involved in mineral homeostasis and physiological performance, as well as acting as a co-factor for many enzymes (Huskisson *et al*., 2007). All the trace elements detected were below the permissible limits of <50 mg/g, with exception to iron which recorded 263 mg/g, 121 mg/g for Akwa Ibom and Cross River states, respectively.

The high concentration of iron for both honey samples could be attributed to the equipment used for processing and storage. Gajek *et al.* (1987) reported that Honey that comes into contact with metal containers or equipment during storage, processing or shipping, may have elevated levels of iron. The number of different minerals and heavy metals in honey is largely dependent on the soil composition, as well as various types of floral plants, because minerals are transported into plants through the roots and are passed to the nectar, and finally into the honey produced from it (Anklam, 1998). Also, the beekeeping practices, environmental pollution, and honey processing also contribute to the diversified mineral content found to be present in honey (Pohl, 2009). Another key trace mineral detected in both honey samples is Manganese, which acts as co-factor for up to 300 enzymes most of which are related to anti-oxidant reactions. Manganese deficiency contributes to aging and age-related disorders (Huskisson *et al*., 2007). Other trace metals noticed in both honey samples include Chromium, Zinc, nickel, Manganese, selenium, and copper, all of which are toxic to human health, and they play important roles as bio-indicators of environmental pollution (Celli and Maccagnani, 2003). All ultra trace elements were also below the permissible limits with exception to titanium which was higher than the recommended value of less than 1 μg/g (78 mg/g, 44 mg/g for Akwa Ibom and Cross River, respectively). Most elements detected from honey sample are useful for good health at optimum concentrations, especially when they originate from plant or organic source, rather than that of inorganic and metallic source. They will have five times the specific gravity of water and become toxic. At this stage they are referred to as heavy metals, which are known to be toxic or poisonous at low concentrations because of their tendency to accumulate in living organism, thereby leading to toxicity in humans. The toxicity occurs due to the inability of the heavy metal to be metabolized by the body, leading to accumulation in human or animal soft tissues without being fully inactivated or destroyed (Ajibola *et al*., 2012). Health problems caused by heavy metals include headaches, metabolic abnormalities, respiratory disorders, nausea, and vomiting. For instance, lead can cause damage to the brain, kidney, nervous system, and red blood cells (Garca-Fernandez *et al*., 1996).

5. CONCLUSION

Honey consumption is associated with various nutritional benefits and therapeutic potential. The biological activity of honey is affected by its complex components. The composition of honey is strongly influenced by both, biotic and abiotic factors, which vary based on geographical and botanical origins. Nevertheless, minerals and heavy metals are minor constituents of honey, and they play vital roles in determining honey quality. Chemical elements in honey samples throughout the world vary in terms of concentrations and are also influenced by environmental pollution. Further studies should be undertaken to help in finding out possible sources of heavy metal pollution and vegetation of the area from where the honey originated.

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