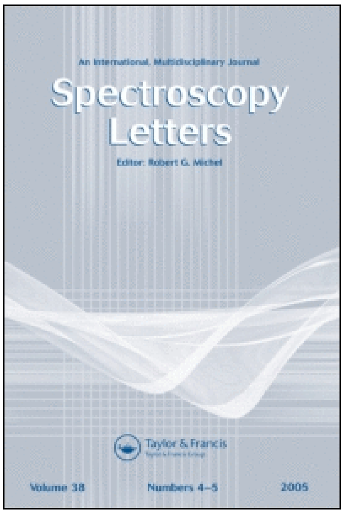

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Determination of Trace Metals in Southern Nigerian Honey by Use of Atomic Absorption Spectroscopy

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ABSTRACT Atomic absorption spectrophotometry (AAS) was employed for the elemental characterization of samples of honey—a multifunctional viscous liquid that is made by honeybees. The honey samples were collected from five different towns in southern Nigeria. Seven elements (Ca, Zn, Fe, Cu, Ni, Ti, and Pb) were determined. Iron was the most abundant element with mean concentration of 224 ppm whereas Pb was the least abundant with mean concentration of 0.62 ppm. The results indicate that honey is rich in essential and beneficial minerals required for healthy growth. The correlation matrix results also show that some of the trace metals determined have common sources or chemical similarities.

KEYWORDS AAS, honey, trace metal

INTRODUCTION

Honeybees, which are social insects, have been producing honey as they do today since at least the Cretaceous period.[1] Honey, a sweet and viscous fluid substance, is made when the nectar and sweet deposits from plants are put together, modified, and stored in the honeycomb by honeybees.[1] On the average, honey is 1 to 15 times sweeter (on a dry weight basis) than sugar.[2] Because of its distinctive flavor, many people prefer it to table sugar and other sweeteners.[3] Honey has attractive chemical properties for baking. It is basically composed of sugar, water, enzymes, minerals, and vitamins.[3] Honey, like milk, is an article of diet whose sole function in nature is food.[4] Honey is also medicinal,[5,6] is used in apitherapy, as an alcoholic beverage (mead, honey wine), and is an excellent natural preservative.[6] It was reported by NHB[6] that liquid honey does not spoil; this is because of its high sugar concentration, which kills bacteria by plasmolysis. The moisture content of honey is too low (14–18%) for natural airborne yeast to survive well in it, and because the moisture content of honey is below 18%, virtually no microbes can reproduce in it.[6] Honey is not always healthful because it is components are gathered from flowers in the wild; situations do arise in which it may be toxic as a result of poisonous substances such as grayanotoxin, the poison tutin, and toxic metals present in

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some flowers.[6] In Nigeria, honey is harvested locally from the wild and from apiculture, which is now a growing industry.

The aim of the current study was to investigate the detection and concentrations of trace metals (Ca, Zn, Fe, Cu, Ni, Ti, and Pb) in some selected honey samples from southern Nigeria using atomic absorption spectrophotometry (AAS) technique.

The atomic absorption spectrophotometer is an instrument that can be used to analyze the concentrations of metals in solution. Sixty-eight metals can be determined directly over a wide range of concentrations from parts per million (ppm) to parts per billion (ppb) levels with good precision, typically better than 1% relative standard deviation (RSD). The instrument is simple and easy to operate.[7]

MATERIALS AND METHODS

Sample Collection and Preparation

Samples of honey were collected from five different locations (Ilara-Mokin, Ile-Ife, Afikpo, Ikot-Ukap-Itam, and Obudu), in southern Nigeria (Fig. 1). To prevent contamination during sampling, transport, and storage, the honey samples were kept in labeled plastic containers, tightly sealed, and taken immediately to the laboratory for analysis.

Sample Digestion

Sample weighing 0.5 g was digested in a 125-mL Teflon beaker (previously soaked in 10% nitric acid for 48 h and later rinsed with double-distilled water) with 3 mL concentrated H_2SO_4 and 5 mL 50% H_2O_2. The Teflon beaker with its content was heated on a hot plate at 70°C for 10 min and the digest turned colorless. After digestion, the beaker was allowed to cool to room temperature. The digest was poured into a 50 mL volumetric flask, and double-distilled water was used to rinse the Teflon beaker into the volumetric flask; the flask was made to the mark with double-distilled water.[8]

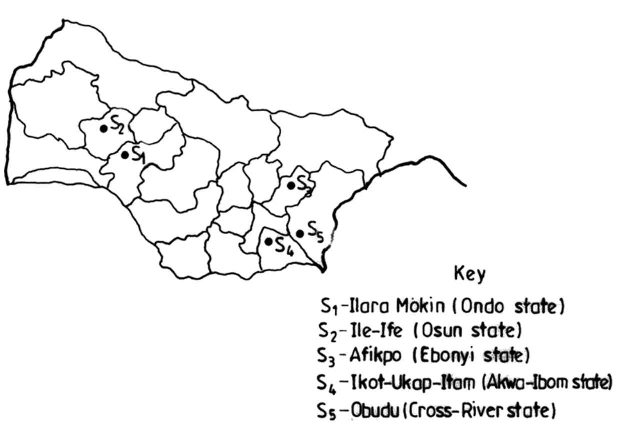

FIGURE 1 Map of southern Nigeria showing the study areas.

Analytical Methods

The analysis of the digested honey samples was performed with a Graphite Furnace Atomic Absorption Spectrophotometer (Chemtech Analytical, UK) at the Centre for Energy Research and Development of Obafemi Awolowo University (Ile-Ife, Nigeria). The instrument was operated as per the instrument's handbook, and calibration was done using mixed calibration standard solution prepared from the pure BDH Analar grade salt of each metal. Data were acquired on a Gateway 2000 PC system using appropriate software. As control, a blank determination was also made following the same procedure described above, and all the reagents used were of BDH Analar grade. Seven elements were determined, namely, Ca, Zn, Fe, Cu, Ni, Ti, and Pb.

RESULTS AND DISCUSSION

The values of the trace metals in southern Nigerian honey determined using AAS are presented in Table 1. Seven metals and their concentrations were determined, and the correlation matrices for the elemental concentrations were calculated using the SPSS program (SPSS Inc., Chicago, IL). The values obtained have the following ranges: Ca (144–270 μg/g), Zn (44.6–113 μg/g), Fe (120–342 μg/g), Cu (13.9–21.0 μg/g), Ni (5.00–13.0 μg/g), and Ti (25.0–71.0 μg/g), and the values of Pb ranged between 0.00 and 0.90 μg/g. Iron was the most abundant metal with mean concentration of 224 μg/g, followed by Ca with mean concentration of 218 μg/g and Zn with mean concentration of 73.6 μg/g, and Pb was the least with mean concentration of 0.62 μg/g. Though there are no significant differences in the concentrations of metals in the honey

TABLE 1 Concentrations of Trace Metals in Honey from Southern Nigeria

		Elemental concentrations (μg/g)							
Honey samples	Sampling town	Ca	Zn	Fe	Cu	Ni	Ti	Pb	$\sum_{(TM)}$
1	Ilara-Mokin	144	82.7	120	19.4	7.00	38.0	0.90	412
2	Ile-Ife	230	113	220	12.3	11.0	25.0	0.70	612
3	Afikpo	270	69.2	312	20.1	5.00	47.0	0.90	724
4	Ikot-Ukap-Itam	240	44.6	342	13.9	9.00	36.0	Nd	686
5	Obudu	204	58.7	124	21.0	13.0	71.0	0.60	292
	Mean	218	73.6	224	17.3	9.00	43.4	0.62	545
	Range	144–270	44.6–113	120–342	13.9–21.0	5.00–13.0	25.0–71.0	ND–0.90	292–724

ND, not detected.

from the different sampling locations, the results indicate that sample 3 has the highest trace metals summation {$\sum_{(TM)}$} of 724 μg/g, followed by sample 4 (686 μg/g), and sample 5 (292 μg/g) has the lowest; this will also be the order of the ash contents because the ash contents are determined by the amount of mineral elements present in honey.[6] These also show that the honey samples are rich in essential minerals that are required for the well-being of humans. Calcium is required for bone formation and development; Fe and Cu are essential to life because they play major roles in blood building and the functioning of critical enzyme systems; and Fe is also needed in small amount for glutathione peroxidase, with its deficiency leading to endemic cardiomyopathy. Copper also plays important roles in normal carbohydrate and lipid metabolisms.[9] Zinc is a multifunctional nutrient involved in glucose and lipid metabolism, hormone function, and wound healing,[9] and it is also essential in proper hair growth.[10] Small amounts of Ni are needed to produce red blood cells.[10] Lead, which is known to be xenobiotic (it is neither essential nor beneficial in human physiology but has detrimental effect to the physiologies of humans and other living organisms[10,11]), has comparatively low concentrations. Comparison of the results with what Asubiojo et al.[12] obtained for Nigerian evaporated milk showed that the southern Nigerian honey is less rich in Ca (218 μg/g) than is Nigerian evaporated milks (Ca mean concentration of 1239 μg/g) but is richer in Zn (73.6 ppm) and Fe (224 μg/g).[13]

In order to test the adequacy of dietary intake of honey for supply of some essential elements, the elemental concentrations of honey was compared with Dietary Reference Intakes (DRIs) (Table 2). It is found that at a daily consumption rate of 100 g/day, the values of the elements Zn, Fe, Cu, and Ni in the honey are within the U.S. recommended DRIs.[14]

The results of correlation matrices for the elemental concentrations of the honey samples are presented in Table 3. Some of the metals are

TABLE 2 Comparison of elemental composition of honey with Dietary Reference Intakes (DRIs): Recommended Dietary Allowances (RDAs) and Adequate Intakes (AIs) for individuals.[14]

				DRI	
Element	Mean	Range	mg/100g	RDA/AI	Tollerable upper intake levels (UL)
Ca	218	144–270	21.8	800–1300	NA
Zn	73.6	44.6–113	7.36	5.00–12.0	12.0–40.0
Fe	224	120–342	22.4	10.0–27.0	40.0–45.0
Cu	17.3	13.9–21.0	1.73	0.44–1.30	3.00–10.0
Ni	9.00	5.00–13.0	0.90	0.30–1.00	0.30–1.00
Ti	43.4	25.0–71.0	4.34	NA	NA
Pb	0.62	ND–0.90	0.062	NA	NA

NA, not available; ND, not detected.

TABLE 3 **Pearson correlation matrix of the analyzed metal**

	Ca	Zn	Fe	Cu	Ni	Ti	Pb
Ca	1.000						
Zn	−0.174	1.000					
Fe	**0.837**	−0.316	1.000				
Cu	−0.262	−0.356	−0.420	1.000			
Ni	−0.153	0.056	−0.442	−0.212	1.000		
Ti	−0.056	−0.547	−0.364	−0.797	0.320	1.000	
Pb	−0.264	0.589	−0.472	0.514	−0.342	0.080	1.000

$n = 5$, $\alpha = 0.05$, $r \geq 0.754$ at 95% confidence interval level. Positively correlated metals are presented in boldface type.

positively correlated whereas some are negatively correlated. In order to determine which of the correlations are *significant* in the statistical sense, the critical multiple correlation coefficient r was obtained from the table of significant values. The critical value r with $n = 5$, $\alpha = 0.05$ is 0.754. Strong positive correlations exist between Fe and Ca ($r = 0.837$) and Ti and Cu ($r = 0.797$), and no strong negative correlation existed between any metals. The above associations can be explained in terms of a common source or chemical similarities.[13] Thus, the positive correlations found between these metals (Fe/Ca and Ti/Cu) could indicate a common source or chemical similarities.

CONCLUSIONS

The AAS technique was used to determine seven metals (Ca, Fe, Cu, Zn, Ni, Ti, and Pb) in the southern Nigerian honey samples. The results indicate that the honey samples are rich in essential elements Ca, Fe, Cu, Zn, and Ni; they also contain low concentration of Pb, which is a well-known xenobiotic, and are also richer in Zn and Fe than is Nigerian evaporated milk. The results also show that at a consumption rate of 100 g/day, the values of the elements Zn, Fe, Cu, and Ni in the honey are within the U.S. recommended DRIs. Strong positive correlations exist between Fe and Ca and Ti and Cu. These associations can be linked to the metals having common sources or chemical similarities. It is interesting to note that in addition to "total" element concentration, many other factors may affect the bioavailability of a particular element, viz., chemical form of the element (organic or inorganic), the oxidation state (e.g., Fe^{+2} or Fe^{+3}, Cu^{+} or Cu^{+2}); source (plant or animal); and the presence or absence of cofactors that affect the absorption.[15] Thus the availability of determined elements in the honey may not correspond with the "total" concentrations of the elements in the honey.

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