Original Research Article

Beeswax as a Bioindicator of Heavy Metal and Pesticide Residue in Commercial Apiaries

ABSTRACT

This study sought to assceess the concentrations of heavy metals and pesticide residues infrom beeswax collected from Awka, Amizi, Ezzamgbo, and Saki. Atomic aAbsorption spectrophotometrySpectrophotometer (AAS) and GC-FID were used tofor analyzesis and quantifyication theof samples. From the study, Bbeeswax from Awka had the highest concentrations of Cadmium and Iron with values (1.211 mg/kg) and iIron (0.193 mg/kg), while the Saki area of Oyo was observed to have the highest concentrationsnumber of Zinc and Chromium (with the concentration of 2.215 mg/kg) and 0.034 mg/kg, respectively. Ezzamgbo had the lowestleast concentration of all the metals analyzed. Generally, there was a significant difference between the total mean concentrations of all heavy metals sampled (P<0.05) and the recommended standard by the FAO/WHO, except forin cadmium (P>0.05). Different mean pesticides residues were checked and Ezzamgbo recorded the highest values of cChlorophyrifos (0.108 mg/kg), dDichlorovos (0.464 mg/kg), dDicophol (0.282 mg/kg), dDieldrin (0.064 mg/kg), pProfeneros (0.321 mg/kg), and T-nonachlor (0.103 mg/kg), and beeswax sampled from Saki had the highest values of dDianzinon (0.003 mg/kg) and eEndosulfan (0.032 mg/kg), while 2_4 DDT (0.243 mg/kg), hHeptachlor (0.321 mg/kg), and lLindane (0.318 mg/kg) were the highest in Amizi. Statistically, there was a significant difference in the levels of the pesticide residue components recovered from the beeswax sampled atin the various locations (at P<0.05), except forin g-chlordane and Profeneros (P>0.05). The results further showed that all concentrations of the various components of the pesticide residues were below the limit recommended limit by FAO, except for 2_4 DDT, Carbofuran, Dichlorovos, Dicophol, Heptachlor and Lindane. The findings of this study suggestposit that while beeswax from Awka and Saki had the highest metal concentrations, Ezzamgbo and Amizi were more contaminated with pPesticide residues.

Keyword: Heavy Metals, Pesticides Residue, Beeswax, Beekeepers

INTRODUCTION

Several insects in nature create wax, but bees and other Apoidea species are responsible for the production of wax, whichthat is greatly appreciated and utilized by humans (Papa *et al*., 2022). Beeswax is produced by the two most widely cultivated bee species, *Apis mellifera* and *A. cerana*. Beeswax is produced by younger worker bees (between the ages of 12 and 18 days, when they have completed their nursing duties) and is a complex material that is available in liquid form (Giampieri *et al*., 2022). Beeswax is preparedmade by mixing beeswax, pollen, and enzymes with resin from tree buds. Therefore, bBeeswax can then be used as a substitute for wax. There are several practical applications offor beeswax, which includinge embalming, the preservation of papyrus, the conservation of artworks, the manufacture of candles and cult artiefacts, andas well as its usage as a medicinal component and adhesive ingredient (Gupta and Anjali, 2023). Also, Iit also acts as a UV radiation barrier to keep the skin from being corroded or scratched, and preventsit keeps moisture from escaping. Additionally, beeswax serves as a source of nest-mate identification clues for honey-bee colonies because of its unique chemical composition (Dumitru *et al*., 2022). Beeswax is commonly used in the food industrybusiness as a food additive and to extend the shelf life of goods, and it is also widely employed in the pharmaceutical and nutraceutical sectors (Gupta

and Anjali, 2023). However,~~Although~~ there ha~~s~~ves been some ~~improvements and~~ concerns regarding the contamination and adulteration of high-quality beeswax.

The health risks associated with beeswax stem from the presence of pesticide residues (including active substances and their metabolites), antibacterial substances (including antibiotics)~~,~~ and heavy metals (Piven *et al*., 2020). Beeswax can~~may~~ be used to detect the presence of metals and pesticides in soil, water, and plants as a bio-indicator of pollution. Owing~~Due~~ to their unique lipid-based chemical composition, beeswax combs act as reservoirs for a variety of environmental pollutants and toxins (Ngat *et al*., 2020; Ćirić *et al*., 2021). The bioactive compounds, minerals, and trace elements that constitute honey products can be contaminated by heavy metals ~~occurring~~ in the environment, which directly affect~~sing~~ the quality of bee products. Heavy metal~~s~~ contamination may disrupt ~~the activity of~~ the bee colony activity. These substances come mainly from the environment, and their~~whose~~ cleanliness depends largely on human activity (Oymen *et al*., 2022). Antibacterial substances are used to prevent and fight diseases in broods and bees, while~~and~~ pesticides are used~~meant~~ to protect crop plants against fungi (fungicides), insects (insecticides), and weeds (herbicides). The use of pesticides in agriculture is essential for~~to~~ obtaining high yields, but results in the contamination of the soil, water, air, and ~~also the~~ flowers from which bees collect nectar and pollen, the natural components of honey (Oymen *et al*., 2022). These agents negatively affect both bees and humans~~people~~, causing changes in their endocrine and nervous systems (Hernández *et al*., 2016). Heavy metals can have a variety of hazardous consequences for consumers and bees and depend on ~~how polluted the~~ air, water, and soil (Ullah *et al*., 2022).

Long-term exposure to high levels of heavy metals may cause kidney damage, ~~high~~ lead levels in the blood, and carcinogenic risks for adults and children (Mao *et al*. 2019). Heavy metals affect bee development, brood rearing, adult bee lifespan, pollen, nectar, ~~and~~ honey storage, and consumption behaviors (Burden *et al*. 2019). Because~~Since~~ heavy metals can accumulate over a long period ~~of time~~, beekeepers regularly remove old, dark, and damaged combs during the active beekeeping season and transport them to the craft unit for processing into fresh wax foundations (Svečnjak *et al*. 2019). Heavy metals are transported to the hive as a result of bee physiological ~~activity~~ or human activity during routine beekeeping procedures (Borsuk *et al*. 2021). This is now one of the most important challenges related~~ing~~ to beeswax quality, accounting for approximately~~around~~ 60% of all the problems. These challenges include ~~assessing~~ the assessment~~extent~~ of the extent~~contamination~~ of beeswax contamination by pesticides and heavy metals. Therefore, this study aimed~~seeks~~ to assess beeswax as a bioindicator of heavy metal and pesticide residues in commercial apiaries.

MATERIALS AND METHODS

Study Area and Duration of study

The study was carried out at the Spring B~~b~~oard Research Centre, Awka, Anambra State, which falls within the geographical coordinates of latitudes of 6.2220° N and 7.0821° E (Longitude) and stretches in an east–west direction along the Enugu-Onitsha express~~-~~way and about 5 km in a n~~N~~orth-s~~S~~outh orientation. It experiences dry and wet seasons, ~~dry and~~ with~~wet season and~~ a~~the~~ mean annual rainfall of 2.169.8 mm and the highest month of rainfall is ~~the~~ September, with ~~having~~ an~~the~~ average temperature of ~~a~~ 29°C and 34°C.

Collection of Beeswax

The honey beeswax sample were collected from four different states in Nigeria: Saki in Saki west L.G.A of Oyo State which fall within the geographical coordinates (Latitude: 8.6033° N, Longitude: 3.1378° E), Ifite Awka in Anambra State (Latitude: 6.2404° N, Longitude: 7.0902° E), Amizi in Abia State (Latitude:

5.4682° N, Longitude: 7.5024° E) and Ezzamgbo in Ebonyi State (Latitude: 6.3994° N and Longitude: 7.9484° E).

Procedures Digestion for Heavy Metal Analysis

Two grams (2 g) of the sample was weighed into a digestion flask and ~~add~~ 20 ml of the acid mixture aqua regia (65 ml concentrated NHO_3,~~;~~ 8 ml perchloric acid, and 2 ml concentrated H_2SO_4). ~~Heat~~ ~~T~~the flask was heated until ~~a~~ clear digestion wa~~i~~s obtained. ~~Dilute~~ ~~T~~the digest was diluted with distilled water to a~~t~~he 100 ml mark.

Working Principle of Atomic Absorption Spectrophotometer (AAS)

The working principle of the a~~A~~tomic absorption spectrometer~~'s working principle~~ is based~~built basically~~ on the sample being aspirated into the flame and atomized when the AAS~~'s~~ light beam from the monochromator is directed through the flame onto the detector, which~~that~~ measures the amount of light absorbed by the atomized element in the flame when ignited. Metal elements on their own have distinct characteristic wavelengths on the source hollow cathode lamp composed of the element/metals to be analyzed. The amount of energy of the characteristic wavelength absorbed in the flame is directly proportional to the concentration of the element in the tested sample. The sample wa~~i~~s aspirated into an~~the~~ oxidizing air-acetylene flame. When an~~the~~ aqueous sample wa~~i~~s aspirated, a~~the~~ sensitivity of~~for~~ 1% was~~absorption is~~ observed. Soxhlet Extraction of oil from the b~~B~~eeswax samples in a 250 ml round bottom boiling flask was dried in an oven at 105-110ºC for about 15 min~~utes, and~~ transferred into a desiccator, and allowed to cool. The 250 ml flask was filled with n-hexane ~~solvent~~. The weighed sample (20 g) was inserted into the thimble of a~~the~~ S~~s~~oxhlet apparatus with cotton wool underneath to serve as a filter. The apparatus was assembled on the boiling flask of the S~~s~~oxhlet apparatus, ~~and~~ allowed to stand on an electric hot plate at temperature ~~of~~ 60-75°C, and allowed to reflux approximately~~about~~ four~~4~~ times for five repeated extractions. The e~~E~~xtract from the flask was collected and emptied into a rotatory evaporator at temperature ~~of~~ 40-60°C to separate the n-hexane solvent from the extracted oil. The extracted oil was then collected and stored in a container.

3.3.1 Preparation of sample for Pesticide Residues

Five milliliter 5 ml of the oil sample was pipetted into a beaker containing 20 ml of n~~N~~-hexane, shaken very well, ~~and~~ poured into a separating funnel, and allowed to stand for 30 min~~utes~~. The N-hexane layer was collected and stored in a sample vial for pesticide residue a~~A~~nalysis using~~with~~ GC-FID.

Quantification with GC-FID

The ~~analysis of~~ PCB profile was analyzed~~performed~~ on a BUCK M910 Gas chromatograph~~y~~ equipped with a flame ionization detector. A RESTEK 15 m~~meter~~ MXT-1 column (15mx 250u mx0.15um) was used. The injector temperature was with split less injection of 2µl of sample and an oven operated initially at 200°C~~-~~, it was heated to 330°C a rate of 3°c min' and was kept at this temperature for 5 minutes, the detector operated at a temperature of 320°C. The PCB components were determined by the ratio between the area and mass of the internal standard and the area of the identified compounds. The concentrations of the different amino acids are expressed in ug/ml and percentages. Fixed Setting: In ~~addition, the~~ injector and detector temperatures were set. ~~The~~ D~~d~~etectors are generally held at the high end of the oven temperature range to minimize the risk of analyte precipitation. All of these parameters were set to the correct values, but double check all the instruments: Buck 530 gas chromatograph was~~were~~ equipped with an on-column, automatic injector, Flame Ionization detector, HP88 capillary column (100m x 0.25µm film thickness~~,~~) CA, USA Detector Temperature A:250°C Injector temperature 22°C Integrator chart speed: 2

cm/min Set the OVEN TEMP to 180C and was allowed to warm up. When the instrument was ready, the "'NOT READY" light wasdid turned off, and can the rRun began. One1 microliter of the sample waswere injected onto column A using the proper injection technique.

Statistical Analysis

The data collected from the study werewas subjected to aAnalysis of variance using the SPSS version software 25, program and the statistical significance was estimated at a 95% confidence level. The sample means were separated using Turkey-HSD test.

RESULTS

Heavy Metal Concentrations of Beeswax Samples Collected From Different Locations

The results of Tthe mean heavy metal concentrations of the four beeswax samples collected from different locations in Nigeria areis presented in Table 1. The table 1 showsed that the zinc concentration was highest in Saki (2.215 mg/kg) andwhile lowestleast in Ezzamgbo (0.320 mg/kg). The cCadmium concentration was highest in Awka (1.211 mg/kg) and lowest inwhile Saki had the least (0.028 mg/kg). However, iron was highest in Awka while (0.193 mg/kg), whereaswhile Ezzamgbo had the lowestleast (0.020 mg/kg). Chromium concentration was the highest in Saki (0.034 mg/kg), while Ezzamgbo had the lowestleast (0.020 mg/kg). Statistically, there was a significant difference in the iron concentrations among the four locations (P<0.05), whereaswhile no significant difference existeds in Znthe zinc, Cd,cadmium and Crchromium (P>0.05). The results in Table 1 further showed that the total mean concentrations of zinc exceeded the permissible limit by the FAO/WHO, while those of Cd, Fecadmium, Criron, chromium and Ninickel were below the recommended standard. There was a significant difference between the total mean concentrations of all heavy metals sampled (P<0.05) and the recommended standard by the FAO/WHO, except forin cadmium (P>0.05).

Table 1: Mean concentrations of heavy metal in beeswax sampled from four locations

	Mean concentration of Heavy metals (mg/kg) ±SD				
Locations	**Zinc**	**Cadmium**	**Iron**	**Chromium**	**Nickel**
Amizi	0.439^{a}±0.211	0.267^{a}±0.071	0.115^{bc}±0.017	0.024^{a}±0.015	ND
Awka	2.046^{a}±1.06	1.211^{a}±0.663	0.193^{c}±0.024	0.024^{a}±0.004	ND
Ezzamgbo	0.320^{a}±0.113	0.444^{a}±0.191	0.020^{a}±0.028	0.020^{a}±0.006	ND
Saki	2.215^{a}±1.207	0.028^{a}±0.025	0.062^{a}±0.005	0.034^{a}±0.004	ND
Total	1.255±1.122	0.487±0.542	0.097±0.071	0.025±0.009	ND
Permissible Limit (mg/kg)	0.3	0.3	5.0	0.10	0.10
Remark	NS	S	S	S	S

Columns sharing similar superscripts are not significantly different at P>0.05
NS= Not satisfactory; S= satisfactory; ND= Not Detected

Pesticide residue in Beeswax Samples Collected from Four Locations in Nigeria

The results of Tthe mean pesticide residues concentrations of the four beeswax samples collected from different locations in Nigeria areis presented in Table 2. Table 2 showsed that the concentration of 2_4 DDT (0.243 mg/kg), hHeptachlor (0.321 mg/kg), and lLindane (0.318 mg/kg) were the highest in Amizi. Aldrin (0.344 mg/kg), carbofuran (0.435 mg/kg), and-g-chlordane (0.059 mg/kg) were the highest in

Awka. The results further showed that Ezzamgbo hadrecorded the highest values of cChlorophyrifos (0.108 mg/kg), dDichlorovos (0.464 mg/kg), dDicophol (0.282 mg/kg), dDieldrin (0.064 mg/kg), pProfeneros (0.321 mg/kg), and T-nonachlor (0.103 mg/kg), whereaswhile beeswax sampled from Saki had the highest the values of dDianzinon (0.003 mg/kg) and cEndosulfan (0.032 mg/kg). Statistically, there was a significant difference in the levels of the pesticide residue components recovered from the beeswax sampled atin the various locations (at P<0.05), except forin g-chlordane and Profeneros (P>0.05). The table 2 further showsed that all concentrations of the various components of the pesticide residues were below the limit recommended limit by the FAO, limit except for 2_4 DDT, Carbofuran, Dichlorovos, Dicophol, Heptachlor and Lindane.

Table 2: Pesticide residue in Beeswax Samples Collected from Four Locations in Nigeria

Locations	Mean concentration of pesticide residue of beeswax sampled from different locations(mg/kg) ±SD				Total	Limit	Remark
	Awka	Amizi	Ezzamgbo	Saki			
2_4-DDT	0.126^{b}±0.001	0.243^{d}±0.002	0.225^{c}±0.001	0.001^{a}±0.000	0.149±0.103	0.01	NS
Aldrin	0.344^{b}±0.063	0.03^{a}±0.001	0.085^{a}±0.015	0.000^{a}±0.000	0.115±0.147	0.2	S
Carbofuran	0.435^{d}±0.002	0.001^{a}±0.000	0.108^{b}±0.001	0.319^{c}±0.001	0.216±0.183	0.06	NS
Chlorophyrifos	0.000^{a}±0.000	0.000^{a}±0.000	0.108^{b}±0.002	0.000^{a}±0.000	0.027±0.050	1	S
Dianzinon	0.000^{a}±0.000	0.000^{a}±0.000	0.000^{a}±0.000	0.003^{b}±0.001	0.001±0.001	0.01	S
Dichlorovos	0.099^{a}±0.002	0.203^{b}±0.014	0.464^{c}±0.047	0.108^{ab}±0.001	0.218±0.159	0.1	NS
Dicophol	0.000^{a}±0.000	0.002^{a}±0.001	0.282^{b}±0.051	0.000^{a}±0.000	0.071±0.132	0.002	NS
Dieldrin	0.016^{a}±0.007	0.030^{b}±0.002	0.064^{c}±0.000	0.012^{a}±0.000	0.030±0.022	0.2	S
Endosulfan	0.005^{a}±0.004	0.000^{a}±0.000	0.000^{a}±0.000	0.032^{b}±0.000	0.009±0.014	5	S
g-chlordane	0.059^{a}±0.068	0.000^{a}±0.000	0.000^{a}±0.000	0.012^{a}±0.001	0.018±0.037	0.1	S
Heptachlor	0.000^{a}±0.000	0.321^{c}±0.001	0.000^{a}±0.000	0.084^{b}±0.000	0.101±0.141	0.01	NS
Lindane	0.002^{a}±0.001	0.318^{b}±0.002	0.000^{a}±0.000	0.000^{a}±0.000	0.08±0.147	0.02	NS
Profeneros	0.154^{a}±0.069	0.150^{a}±0.174	0.321^{a}±0.000	0.235^{a}±0.001	0.215±0.103	0.5	S
T-nonachlor	0.000^{a}±0.000	0.011^{a}±0.000	0.103^{b}±0.009	0.013^{a}±0.000	0.032±0.044	0.04	S

Rows sharing similar superscripts are not significantly different at P>0.05

NS= Not satisfactory; S= satisfactory

DISCUSSION

One of the most prevalent heavy metals in the Earth's crust is, zinc (Zn), which is said to be a vital trace metal requiredneeded for life's development. It is a cofactor of important proteins and mediates the control of severala number of immunomodulatory processes (Hussain *et al*., 2022). Zinc is necessary for many body processes, but too much of it has also been linked to changes in the immune system and gastrointestinal flora changes (Skalny *et al*., 2021). Higher amounts of Znzinc can be harmful to cells, which frequently leads to the disruption of vital biological processes by mismetallating with other metals to block protein thiols (Marchetti, 2013; Chandrangsu *et al*., 2017). All of the beeswax samples in this study contained zinc, but Saki appeared to have the highest concentration (2.215 ±1.207), followed by Awka (2.046 ±1.06). This is higher than that reported by Omran *et al*. (2019), who reported that the zinc contents ranged from 0.185 to 0.241 mg/kg. The highestgreatest Zn values in the four regions were 3.975, 8.266, 7.052, and 1.122 (mg/g), according to Aljedani *et al*. (2020). Except for the Zn concentration (1.122 mg/g) in the fourth region reported by Aljedani *et al*. (2020), which wais lower than the maximum Zn concentration in this study, the Zn concentration reported by Aljedani *et al*. (2020) was greater than the highest Zn concentration observed in this study.

The mean iron level in the current study was between 0.193 to 0.020 mg/kg, which is lower than the range of 2.068 to 5.041 ppm reported by Hassona *et al*. (2023). In aAdditionally, the iron level was also 56.470 – to 285 ppm (Gajger *et al*., 2019). The Fe concentrations from the several ambient sources ranged between 1.080 and 334 µg/g (Formicki *et al*. 2013) and between 5.972 and 18,516 mg/g (Aljedani, 2020). All of these valueswhich exceeded the iron content found in our investigation. Fe is necessary for the synthesis of hemoglobin, the transportation of oxygen, and the action of a number of enzymes. IronFe metabolism abnormalities, which can range from anemia to ironFe overload and possibly neurological diseases, are among the most common ailments in humans (Abbaspour *et al*. 2014).

Although Cdcadmium occursis naturally occurring in the environment, it is also produced by industrial and agricultural processes, such as smelting copper and nickel, burning fossil fuels, and making phosphate fertilizers, PVC products, and color pigments (Gnechi *et al*., 2020). CdCadmium exposure mostly occurshappens through the consumptionconsuming oftainted food and water, but it can also occurhappen through inhalation and smoking (Taha *et al*., 2018). CdCadmium is considered carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2023). Numerous cancers, including those of the liver, kidneys, breast, lung, and prostate, are associatedmay be linked withto exposure to this metal (Gnechi *et al*., 2020). In this study, cadmium was found in every sample, with Awka having the highest concentration (1.211 mg/kg) of all of the study samples. Theis study's maximum Cdcadmium concentration in this study was lower than that reported byof Aljedani *et al*. (2020), who found a maximum Cdcadmium concentration of 0.075 mg/kg. Additionally, the Cd content found in this study was higher than the Cd value (0.194) mg/kg) reported by Omran *et al*. (2019). The city's location, which includes multiple main roads with piles of corroded metals from accident vehicles and mechanical shops, may be linked to the high content of cadmium content. This could be the cause of the metal poisoning inof the honey. No Nnickel was not found in any of the samples; this could be because materials that could pollute theose locations are not easily manufactured there.

The study's findings revealed that Ezzamgbo had the highest levels of T-nonachlor (0.103 mg/kg), pProfeneros (0.321 mg/kg), dDieldrin (0.064 mg/kg), dDichlorovos (0.464 mg/kg), dDicophol (0.282 mg/kg), and cChlorophyrifos (0.108 mg/kg), all of which were equal to the EU threshold value. The range of atrazine results obtained wasfall within the range reported by (Blasco *et al*., 2003). Farmers in Ebonyi State frequently use these herbicides, particularly in Ezzamgbo, where rice and cassava are produced in largeconsiderable quantities. All other results exceeded the European Union's maximum pesticide residue level of 0.01 mg‡ kg−1 in foods. In Ngwa village, lindane is a pesticide that is frequently used on plants and to control insects in both urban and rural regions. All of the results were higher than the 0.01 mg·kg−1 recommended bythat the European Union recommends, which suggests that they were used excessively on farms and have the potential to endanger ecosystem health. Report on analysis of 2_4 DDT (0.243 mg/kg), hHeptachlor (0.321 mg/kg), and lLindane (0.318 mg/kg) were the highest in Amizi.

CONCLUSION

Contamination of beeswax by pesticides isare very harmful to the body. Contamination withWhen beeswax canare contaminated, it could pose a threat to both bees and humans. From this study, it is evident that heavy metals are prevalent in Awka and Saki which is predisposed to metals could be due to much industrialization, Beeswax from Ezzamgbo were having least contraction of metals. Ezzamgbo showedhad the highest concentration of pPesticide concentration. This may have been caused by much agricultural practices inof which the area is known for among the four locations. The government should develop adequate measures to mitigate the issue of pollution caused by pesticides.

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