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Original Article

Assessment of honeybees and bee honey as bioindicators of environmental pollution

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ABSTRACT

Honeybees, *Apis mellifera* L. is of a major advantage as a bio-indicator and is increasingly used because it meets the criteria for selecting bio-organisms for environmental pollutant assessment. The levels of Manganese (Mn), Cadmium (Cd), Lead (Pb), Nickel (Ni), and Chromium (Cr) were analyzed by flame Atomic Absorption Spectrophotometery in honeybee and fresh bee honey samples randomly collected from five locations (Idi-Ayunre, Otu, Owotoro, University of Ibadan, and Atan) in areas of varying degrees of pollution within Oyo State (Nigeria) to determine the potential of honeybees and bee honey as bio-indicators of heavy-metal environmental pollution. The concentrations of the analyzed heavy-metals in the samples significantly increase ($P < 0.05$) as the levels of pollutants increases in the environment (pollution gradient). The result indicated that the concentrations of Mn, Cd, Pb, Ni, and Cr in both honeybee and bee honey samples from industrial areas and vehicle combustion effluent locations were significantly higher than the values obtained from the other locations in areas of farm land and human residency. Cd occurred in low concentration in all samples while honey samples from Atan were free from Cr due to low pollution source ($P < 0.05$). Significant positive association existed between the heavy-metals in the honeybees and those found in the honey samples analyzed. The heavy metals found in the honey from industrial areas and vehicle combustion effluent areas (highways) were possibly from the honeybees. Correlation analysis of the heavy-metals both in bee bodies and bee honey showed a common origin of majority of the studied metals. Element concentrations in the honey under study were within the safety baseline levels for human consumption recommended by FAO/WHO.

Keywords: Honeybee, bee honey, bioindicator, heavy metals, pollution gradient

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INTRODUCTION

Metals present in the natural environment can come from both natural and anthropogenic sources which are discharges of municipal and industrial wastewater, mining, and metallurgical industry dust from urban streets, road transport, and agriculture.[1,2] Bio-indication is an old method of assessing the state of the natural environment using living organisms such as Mussels (*Dreissena polymorpha*), herrings (*Clupea harengus*), partridges (*Alectoris rufa*), cattle, fox, and honeybees.[3]

Bees occur in large population of at least 1000 worker bees and their presence cannot be limited to only a single habitat. A single bee is estimated to forage on plants up to 12 km, depending on food availability.[2] It can forage on at least 1000 flowers/day from which the honey produced daily can be considered the outcome of at least 1,000,000 plant interactions.[4] Due to the fact that their existence is inextricably linked with their environment, their forage area is effectively sampled for trace elements, and the concentration of heavy metals in honey reflects levels in the foraged area.[5] Honeybees are able to collect and accumulate bioavailable contaminants in "air, water, and soil" in the environment during their foraging flights.[6] They move from flower to flower, touch branches and leaves, drink water from pools, and thus allow their hairy bodies to collect aerosol particles which enable them to accumulate impurities in its body.[3] These, therefore,

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make honeybee fits for selection as a biological organism to indicate and estimate the level of environmental pollution.[7] It has been affirmed that *A. mellifera* are commonly used as test organism in ecotoxicology studies because they are powerful unbiased samplers, easy to identify, easy to rear due to relatively short lifecycle as well as easy to manipulate and handled for laboratory chemical analysis.[8]

High concentrations of heavy metal pollutants in bees' environment accumulate in the body tissues of honeybees, causing disturbances in their cellular activities and subsequently leads to high mortality among them.[9] Despite the great nutritional, medicinal, and cosmetic importance of honey to man, the presence of accumulated heavy metals in the body system of honeybees as a result of environmental contamination with heavy metals can be transferred into the raw honey produced.[10] The potential use of honeybee and honey as bio-indicator of heavy-metal pollutant in the environment has been studied extensively by scientists in various countries such as Turkey, Egypt, and Burgaria while such information is insufficiently available in Nigeria.[2,10-12] The aim of the study is to evaluate the potential of Nigerian *Apis mellifera* L. and bee honey as bio-indicators of metals in locations with varying degrees of pollution in Oyo State, Nigeria.

METHODS

Study Area

The study was carried out at five independent well managed old commercial apiaries sites free from hive pests and diseases with aggressive colonies located along varying degrees of pollution gradient within Oyo State [Figure 1]. The apiaries were located in rain forest zone characterized by dense vegetation, rich in species diversity, long rainy season with heavy rainfall of more than half of the year, and a mean annual temperature (22–25°C).

Sample Collection and Preparation

In 2018 and 2019, adult honeybee workers *Apis mellifera* L. (Hymenoptera: Apidae) and freshly harvested bee honey samples were randomly collected from the apiaries located in Idi-Ayunre, Otu, and Owotoro (heavily polluted areas) Crop Protection apiary, University of Ibadan and Atan (semi-polluted areas) without prior smoking of the hives [Figure 1 and Table 1]. The pollution ratings were represented by numbers: (1) Low polluted area; (2) slightly polluted area; (3) moderately polluted area; (4) highly polluted area; and (5) extremely high polluted area. Three colonies (bee hives) randomly selected from each of the location were used for the study. Adult worker bees (ten individuals) landing on the board at the hive flight entrance were collected in three attempts per hive and stored separately into a well labeled new sterile specimen plastic bottles with tight fitting lids. Fifty grams of honey sample was also pressed out from three randomly selected capped honey combs per hive into well labeled sterile plastic specimen bottles with tight fitting lids. A total of 450 honey bee workers were collected and stored at −20°C while their bee honey samples were stored at room temperature until analysis.

Metal Analysis

The honeybee samples were dried at 45°C, homogenized by grinding, and carefully mixed in the laboratory. Representative 0.5 g sample of both honey and the grinded bee samples were treated separately with 2 ml of HNO_3 (65%) (Merck,

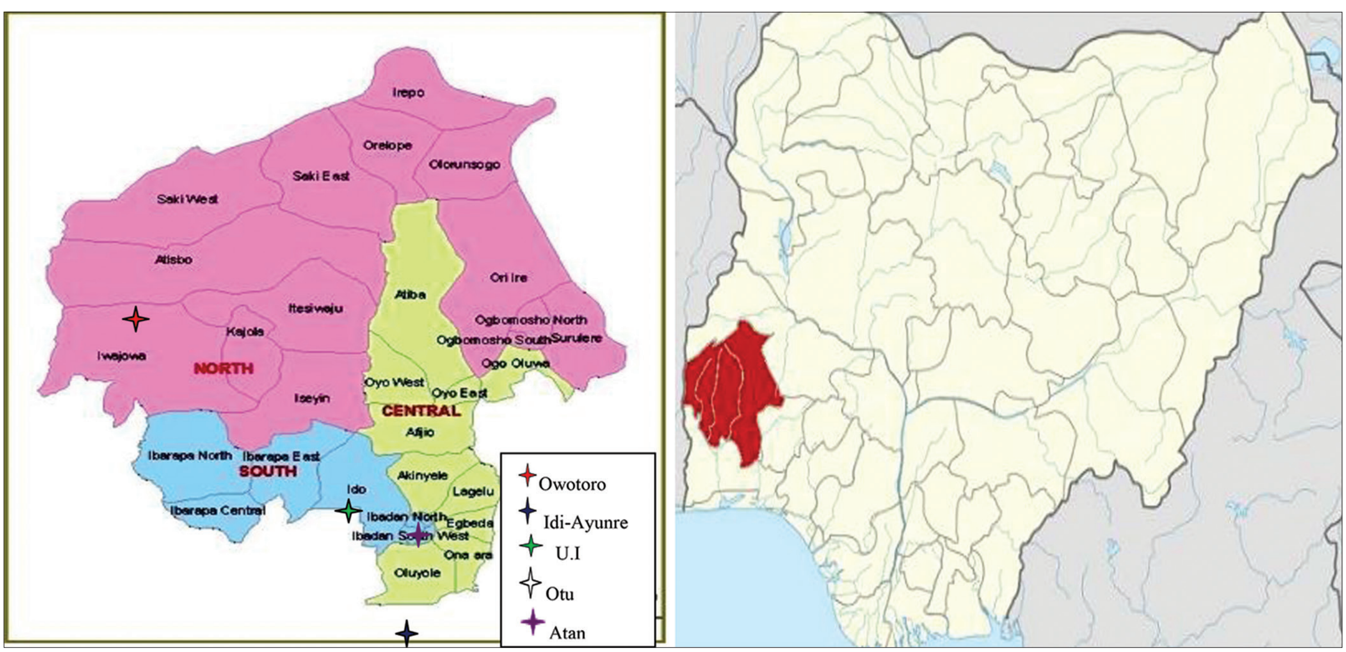

Figure 1: Locations of the apiaries on the map of Oyo State, Map of Nigeria showing the location of Oyo State

Darmstadt, Germany) and 0.5 ml of H_2O (30%) (Merck, Darmstadt, Germany) and then subjected to a microwave-assisted acid digestion device (Milestone, MLS 1200 Mega). The concentrations of heavy metals (Mn, Cd, Pb, Ni and Cr) were determined in the digested samples of honeybees and bee honey using flame Atomic Absorption Spectrophotometer (Analyst 200, Perkin Elmer, USA) according to AOAC procedures in the laboratory of Chemistry, University of Ibadan.[13] The levels of metals in honeybees were calculated based on dry weight while the honey was calculated on wet weight basis. All the examinations were replicated in triplicate and the blank samples were used to ensure that the obtained results for each analyzed metals were within the correct range.

Data Analysis

The results obtained were statistically processed on Microsoft Office Excel 2019 and all the calculations were performed using mean values of three replicates. The normality for the data distribution was determined by Shapiro–Wilk test and Pearson correlation analysis was carried out at the level of $P < 0.05$ to determine the relationship and association between data on individual heavy-metals in honeybee samples and those found in bee honey samples. Descriptive statistics such as charts were used to express the influence of degree of pollution gradients on the concentrations of analyzed metals in both bees and honey samples.

The results obtained were also compared for interpretation with reference permissible levels of each individual element in honey and human food according to FAO/WHO standards.

RESULTS

The study revealed the presence of the analyzed heavy-metals in both honeybee and bee honey samples [Figures 2 and 3]. The concentrations of Mn, Cd, Pb, Ni, and Cr concentrations in honeybees and bee honey increased significantly with the pollution gradient levels in the study areas and reached the peak at Idi-Ayunre (urban and industrial area) ($P < 0.05$).

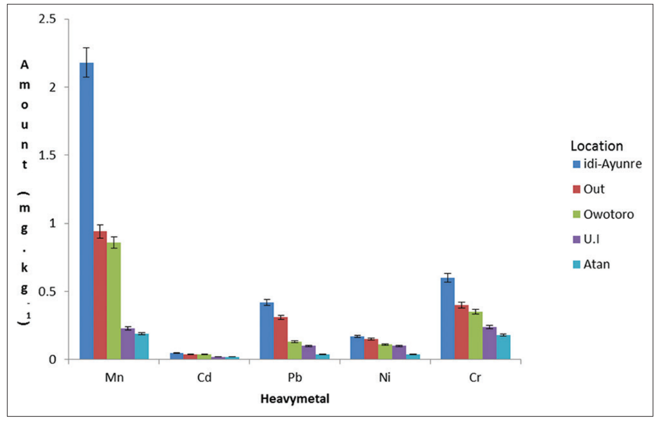

Figure 2: Heavy metals concentrations (mg/kg) in worker honeybees (Apis mellifera L.) collected from commercial apiaries located in polluted areas of Oyo State

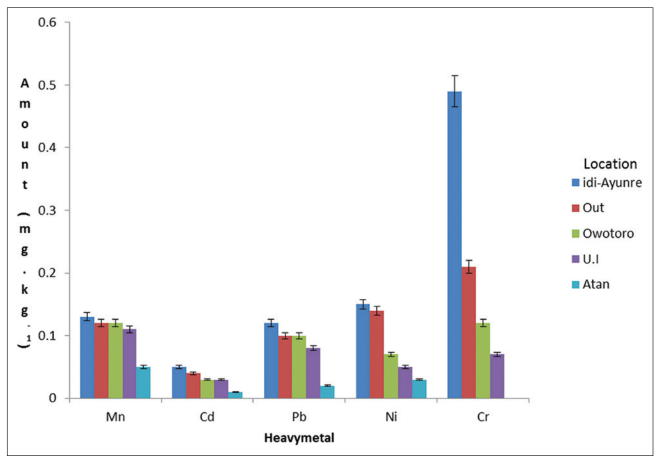

Figure 3: Heavy metals concentrations (mg/kg) in bee honey samples collected from commercial apiaries located in polluted areas of Oyo State

Table 1: Description of locations studied for environmental pollution assessment in Oyo State, Nigeria

Location	GPS coordinates	Pollution source	Pollution rating
Idi-Ayunre	7°13' 59.99" N, 3° 52' 0.00" E	Exhaust fumes from vehicles on highway and industrial plants, chemical waste from beverage and plastics industries, chemical waste from Procter and Gamble industry	5
Otu	8°12' 25.74" N, 3° 31' 49.04" E	Exhaust fumes from vehicles on highway and intense human activities	4
Owotoro	8°24' 11.99" N, 3° 15' 2.08" E	Poultry waste, dump site, and pesticides use on farms	3
Crop Protection apiary, University of Ibadan	7° 27' 2.81" N, 3° 53' 58.96" E	Office waste and household waste	2
Atan	7°34' 10.52" N, 3° 53' 4.09" E	Herbicides use on oil palm and plantain farm only	1

5 – most heavily polluted areas, 4 – heavily polluted areas, 3 – moderately polluted areas, 2 – lightly polluted areas, 1 – less polluted areas.dapted from Pitan (2008).[14]

Honeybee samples collected from Idi-Ayunre (industrialized area) had the highest significant value (2.18 mg/kg) of manganese compared with all other locations while the manganese content of Otu (highway and anthropogenic areas) (0.94 mg/kg) and Owotoro (poultry waste, dump sites, and farms) (0.86 mg/kg) was statistically similar. Mn found in bee bodies samples from University of Ibadan (office area) (0.23 mg/kg) was significantly higher than samples from Atan (farms) (0.19 mg/kg) [Figure 2, $P < 0.05$]. The bees from Idi-Ayunre (Industrialized area) had the highest significant concentration (0.60 mg/kg) of Cr while the lowest value was recorded in samples from Atan (farm) (0.18 mg/kg) [Figure 2]. Honey samples from Idi-Ayunre (Industrialized area) also had the highest concentration of Cr (0.49 mg/kg) while samples from Atan were free from Cr contamination [Figure 3]. The results of this study showed that the Lead (Pb) content in all the honeybee samples varied significantly within the locations with concentrations in samples from Idi-Ayunre significantly higher (0.42 mg/kg) than bees in Otu (Industrialized areas) (0.31 mg/kg) and the least recorded in samples from Atan (farm) (0.04 mg/kg) [Figure 2]. Honey samples collected from Idi-Ayunre were significantly higher in Pb content (0.12 mg/kg) compared to samples from Otu (Industrialized areas) (0.10 mg/kg) while honey samples from Atan (farm) had the lowest Pb content (0.02 mg/kg) [Figure 3]. Ni and Cd content in the bee samples also varies significantly within the locations. The lowest content of Ni in both samples of honeybees and bee honey was recorded in Atan location at 0.04 mg/kg and 0.03 mg/kg, respectively, while the highest concentration of Ni in samples of honeybees and bee honey was recorded in Idi-Ayunre at values of 0.17 mg/kg and 0.15 mg/kg, respectively.

The concentration of Cd in the bee and bee honey samples was also significantly different among all the studied location [Figures 2 and 3].

The Pearson correlation analysis showed that most of the individual heavy-metal concentrations in all honey bee samples were highly dependent on one another [Tables 2 and 3]. Cd, Pb, and Cr showed high positive level of correlation with Mn ($r > 0.92$) in the honeybee samples, while there was significant positive relationship between Cd and Pb, Ni and Cr ($r > 0.89$) [$P < 0.05$, Table 3]. Similarly, the presence of Pb in the honeybee was dependent on Ni and Cr ($r > 0.93$), while a significant association was found between Cr and Ni content in the bee bodies samples. A significant positive level of dependence (from $r = 0.89$-0.99) was also observed among the metals found in the honey samples along the five pollution gradients studied [$P < 0.05$, Table 3]. The study indicated a positive significant high level of dependence between Mn and Cd, Pb content in the bee honey samples ($r > 0.96$). Cadmium metal also showed a strong positive dependence on Pb content ($r > 0.89$) while similar association exists between Ni and Cr content in the bee honey samples. When comparing the Cr content of honeybee and bee honey samples collected from the different locations, the concentration of Cr were significantly different from each other ($P < 0.05$).

According to the pollution standards, the values of concentrations of Mn, Cd, Pb, Ni, and Cr Pb recorded in both honeybees and honey used in the study were within the standard level indicated by Food and Agriculture Organization of the United Nations.[15]

DISCUSSION

According to the study, honeybees and honey are good potential bioindicators of environmental contamination with metals. The high accumulation of Mn, Cd, Pb, Ni, and Cr metals in honeybees and bee honey of the locations can be attributed to exhaust fumes from the vehicles on the highway, industrial plants, and chemical wastes produced by industries in urban and industrial areas of high pollution gradient levels. Pollutants from exhaust fumes in vehicles, industrial emissions as well as chemical waste scattered in the environment are easily picked by forager bees during flight, feeding, and water drinking. The average levels of metals occurred in the following declining order in the bee samples: Mn > Cr > Pb > Ni > Cd.

Manganese is an essential element in living organism's metabolism but highly toxic in high doses. The presence of Mn in the study area probably originates from transport fumes and industrial emissions. Highest level of Mn was similarly reported in tested bee samples from Stara Zagora city (43−114 mg/kg) and urban areas of Serbia (34–90 mg/kg).[12,16] Chromium is a major problem in world environmental pollution that comes mostly from anthropogenic sources, metallurgical and chemical industry, cement, plastics, dyes, and detergents industries. Intense human activity positively influence increases in the level of Cr in the bodies of bees and honey samples in the study.[17] In a similar study, honeybee samples from the airport area of

Table 2: Pearson correlation (R) between the heavy metals in honeybee and bee honey samples collected from commercial apiaries in five pollution gradient areas in Oyo State

Heavy-metals	Honeybee/Honey
Mn	0.66
Cd	0.84
Ni	0.94*
Pb	0.79
Cr	0.98**

*Correlation is significant at the 0.05 level two-tailed; R=Correlation coefficient.*Correlation is significant at the 0.01 level two-tailed

Table 3: Pearson correlation (R) between the heavy metals in honeybee and bee honey samples collected from commercial apiaries in five pollution gradient for achieved dependencies

Sample	Heavymetals	Mn	Cd	Pb	Ni	Cr
Honeybee	Mn	1.00	0.96**	0.92*	0.83	0.99**
	Cd	0.96**	1.00	0.92*	0.89*	0.98**
	Pb	0.92*	0.92*	1.00	0.93*	0.96*
	Ni	0.83	0.89*	0.93*	1.00	0.91*
	Cr	0.99**	0.98**	0.96*	0.91*	1.00
Bee honey	Mn	1.00	0.96*	0.73	0.99**	0.68
	Cd	0.96*	1.00	0.87	0.98**	0.84
	Ni	0.73	0.87	1.00	0.80	0.89*
	Pb	0.99**	0.98**	0.80	1.00	0.77
	Cr	0.68	0.84	0.89*	0.77	1.00
	Mn	1.00	0.96*	0.73	0.99**	0.68

*Correlation is significant at the 0.05 level two-tailed; R=Correlation coefficient. **Correlation is significant at the 0.01 level two-tailed

Brest district, Belarus recorded an average of 0.24 mg/kg Cr concentration and samples from agro-forestry area recorded Cr concentration of 0.19 mg/kg.[16] Apiaries located close to highways and industries stand the risk of contamination by Pb as fumes from gasoline and internal combustion engines in vehicles and industrial machines are the main source of contamination.[18] In polluted areas, lead can contaminate air, nectar, soil, water and plants, and later enter humans through food chain.[19] In a similar study, high lead content was reported in bee product samples collected from highly polluted areas in Europe.[20] The low concentrations of Ni and Cd in the bee samples among all the studied locations can be attributed to low sources of this contaminants in the apiaries environment while the absence of Cr in bee honey samples of farmlands and forests areas can be attributed to lack of industry, mining, automobile exhaust gases, and almost non existing anthropogenic activities. In a similar study on the honey samples in the south and east region of Turkey, a very low concentration of Cr was reported due to the uncontaminated environment.[21] Metals Cd and Ni are considered bioindicators for honey contamination in view of its toxicity for human.[22] Cadmium (Cd) is an element whose source is industrial pollution which might contaminate the soil or air and from there passed into plants through the root system to the nectar in flowers.

The concentration of the heavy metal in bee honey samples from Idi-Ayunre and Otu (urban and industrial areas) increased in the order; Cr > Ni > Mn > Pb > Cd while bee honey samples collected from Atan, University of Ibadan apiary and Owotoro had the lowest concentrations of all the metals analyzed. Similarly, other studies have reported higher levels of heavy-metal concentrations in samples of *A. mellifera* collected from hives placed in the inner city or in the surroundings of industrial areas or adjacent to busy roads.[4,11,23]

Higher amount of Mn, Cd, Pb, Ni, and Cr reported in this study is similar to previous report in bee honey samples from industrial areas of Eastern Slovakia, Serpentine areas in Eastern Rhodopes Mt in Bulgaria, Argentine, and Hungary.[20,24-26] The correlation analysis results suggested that Cr and Ni in the honeybees were responsible for those found in their honey product. An association has been reported between heavy metal concentration in honey and honeybee body due to the fact that honeybee process their foraged nectars and pollen into honey which might have undergone purification during the process.[27] However, Mn, Pb, and Cd content had no significant association in both honeybee and bee honey, suggesting that the metals were from some other sources in the study areas. A close correlation has been reported between the accumulation of metals in soil and plants as well as metal contents in honeybees and bee products samples of Brest district, Belarus.[28] These dependence shows that the metals found in the honeybee and bee honey had a common source (air, soil, plants, or water) in the environment. High level of dependence has been reported between Cu, Zn, Mn, and Fe analyzed in honeybee samples in a similar study conducted at Bialystok.[2]

CONCLUSION

The results of this study showed the presence of Mn, Cd, Pb, Ni, and Cr metals in both honey bees and bee honey samples analyzed, thus providing evidence that honeybees and bee honey have the potential of detecting and monitoring environmental toxic metal contamination. The study also established that concentrations of the heavy metals were dependent on the level of pollution in the pollution gradient among the five different locations studied. However, hives placed around highways and industries have high concentrations of the heavy metal compared to other locations and majority of the studied metals also showed a common origin.

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