Assessment of heavy metals profile in feathers of birds from Kano metropolis, Nigeria, in 2019

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Abstract
Background: Contamination by heavy metals has been a major threat locally, regionally, and globally because it affects the structural and functional properties of an environment. The aim of the study was to assess the concentration of heavy metals (Pb, Cd, Cr, and Mn) in the feathers of bird species sampled from three areas in Kano metropolis, Nigeria.

Methods: Fifteen samples of birds' feathers were collected from industrial, residential, and commercial areas using mist-nets which were randomly placed to catch passerine birds, early in the morning from 6a.m. to 8a.m. The collected samples were then digested and subjected to atomic absorption spectrophotometry to determine Pb, Cd, Cr, and Mn.

Results: The highest concentrations of heavy metals in the birds' feathers were observed in site A (Sharada) followed by site B (Kurmi market) and site C (Rijiyar Lemo), however, the difference in the concentrations of heavy metals between the study areas was not statistically significant (P > 0.05). Pb had the highest concentration followed by Cd, Cr, and Mn. The highest concentrations of metals were found in the feathers of cattle egret (Pb=89 ppm, Cd=45 ppm, Mn=0.022 ppm), mallard drake (Pb=78 ppm), hen (Cd=41 ppm), duck (Cr=2.75 ppm), rooster (Mn=0.024 ppm), and guinea fowl (Cd=41 ppm), respectively.

Conclusion: The birds' feathers contained various levels of heavy metals which greatly reflect the nature of the sites. Sharada industrial area (site A) recorded the highest concentrations of metals indicating slight metal contamination in the birds' feathers especially in site A.

Keywords: Birds, Feathers, Metals, Monitoring, Pollution

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Introduction
Heavy metals contamination in the world has been considered as a major threat because it affects the properties of an environment both structurally and functionally (1,2). The toxicity profile of such metals influence the performance of many crucial organs such as the central nervous system (3). Many studies have been conducted to ascertain heavy metals occurrence, accumulation, and distribution in addition to other pollutants from natural and anthropogenic activities (1,4). Living organisms at individual and population levels have been used as bioindicators at various trophic levels to obtain information on the extent of exposure, contamination, and effects of chemical pollutants (5,6). Various species of bird including heron and egret have been widely used in biological monitoring of environmental pollution because of their position in the food chain. Thus, having occupied a high level in the food chain makes them exposed to a wide range of pollutants (7,8). Several studies have evaluated the accumulation of heavy metals in organs of bird species (5,9) including eggs (4,10), eggshells (11), feathers (10,12), and prey samples (13). The studies have been performed on various bird species and have generated valuable data on the spatial and temporal distribution of heavy metals (4). One of the non-destructive biomonitoring tools is bird's feathers which have been known to have a potential in assessing the health of local ecosystems. Recently, several studies have used hair and feathers as nondestructive techniques to determine the concentrations of heavy metals and organic pollutants (8). Hence, biomonitoring of environmental pollutants including heavy metals in some species from a designated area could be used to assess the health of the species, as well as the concentration of pollutants.
in their environment (14). There have been very little efforts and information on environmental pollution in Kano possibly because of less biological monitoring strategies. This is despite the huge population of the state with enormous industrial and related activities taking place in the state which produce large tons of waste and pollutants. These necessitate the need to narrow the gap by employing some biological indicators of environment to assess the condition and contamination or pollution status. Kano is the most populous and densely populated state in Northern Nigeria. The state has many settlements and localities that fall under the categories of residential, commercial, and industrial areas. The aim of the study was to assess the concentration of heavy metals including lead (Pb), cadmium (Cd), chromium (Cr), and manganese (Mn) in the feathers of bird species sampled from three areas in Kano metropolis.

Materials and Methods

Study area
The study was conducted in Kano State located in the northern part of Nigeria. Kano State has an estimated land area of 43 070 km$^2$ and population of 9 383 682 million people according to 2006 census. It is located between longitude 100°25’ N and 130°53’ N and latitude 7°10’ E and 100°35’ E, with an altitude of 400-800 m above the sea level (Figure 1). The birds’ feathers samples were collected from three different urban areas of Kano including Sharada, Rijiyar Lemo, and Kurmi market. The areas were selected based on the peculiar and characteristic activities which were industrial (Sharada), residential (Rijiyar lemo), and commercial (Kurmi market), respectively, as prescribed by the State Ministry of Land and Survey. The study was done between September and October 2019.

Sample collection
Fifteen different samples of birds’ feathers were collected from the sampling sites using mist-nets which were randomly placed to catch passerine birds, early in the morning between 6 AM to 8 AM. The mounted mist-nets were checked for possible catch after every 20 minutes. Body feathers were collected from the birds captured including insectivorous, omnivorous, granivorous, and nectarivorous birds. Ringing and morphometric measurements were not recorded so as to prevent further stress on the captured birds. Few feathers were collected from each bird and they were immediately released (15). The birds images were captured using a digital camera and identified using identification guide of Tropical Wildlife Field Guide: Birds A4 (16).

Reagents and chemicals
Glasswares and plastics were washed, rinsed many times with tap water, and then, soaked in 5% nitric acid solution for 24 hr followed by rinsing with deionized water (17). Analytical grades of nitric acid (65%, Sigma Aldrich) and perchloric acid (70%, Sigma Aldrich) were used for sample preparation. Standard solutions for calibration of Pb, Cr, Cd, and Mn were prepared from 1000 mg/L standard stock solution of GFS Fishers’ AAS Reference Standard. All the solutions were prepared in distilled water. Dilution correction method was applied for samples diluted or concentrated during analysis. These stock solutions were serially diluted to provide concentrations as presented in Table 1 (17).

Sample treatment and analysis
After collecting the body feathers from the birds, they were washed using alternating distilled water and acetone three times to remove possible contaminants. The samples were then air-dried before oven drying at 105°C for 2 hours. The feathers were subsequently cut into smaller pieces using a stainless steel scissors to facilitate the acid digestion process. The acid composed of a mixture of concentrated perchloric acid (HClO$_4$) and nitric acid (HNO$_3$) with the ratio of 1:4 by volume (aqua regia). One gram of the feathers samples was added to the acid mixture for complete digestion. The digestion was stopped when

| Metals | Calibration Concentrations (ppm) | Wavelength (nm) | Slit Width (nm) | Detection Limit (ppm) | Lamp Current (mA) | Linear Range (mg/L) | Flame Type (color) | Recovery (%) |
|--------|---------------------------------|-----------------|-----------------|------------------------|------------------|---------------------|------------------|--------------|
| Mn     | 0, 0.001, 0.01, 0.1             | 279.5           | 0.7             | 0.001                  | 6.5              | 2.30                 | A-A, leanblue   | 75           |
| Cd     | 0, 15, 30, 45                   | 228.80          | 0.7             | 0.01                   | 2.00             | 2.00                 | A-A, leanblue   | 73           |
| Cr     | 0, 1, 2, 3                      | 357.90          | 0.7             | 0.01                   | 2.00             | 5.00                 | A-A, richyellow | 89           |
| Pb     | 0, 30, 60, 90                   | 283.30          | 0.7             | 0.08                   | 5.00             | 20.00                | A-A, leanblue   | 90           |
a colourless solution was obtained and it was evaporated to dryness. The resulting solution was diluted to 25.0 mL with deionized water. The solutions were allowed to cool, filtered by Whatman filter paper 42 into a 100 mL calibrated flask, and were diluted up to the mark. The sample solution was analyzed for Cd, Cr, Pb, and Mn using Flame Atomic Absorption Spectrophotometer (210 VGP, Buck Scientific, East Norwalk, USA) (17).

Data quality assurance/quality control (QA/QC)
Method validation was performed by assessing several analytical figures of merit including linearity and range, precision, limit of detection (LoD), limit of quantification (LoQ) and accuracy, according to International Conference on Harmonisation (ICH) (18). Sensitivity of analytical methods was done according to the ICH (18) and described by the LoD and LoQ (19,20). The precision was measured as relative standard deviation of the concentration in the study. The measurements were done under conditions of repeatability and intermediate precision (19). The accuracy of the process was determined by calculating recoveries of Cd, Cr, Pb, and Mn. According to the ICH (19) for determining the recoveries, the spiking technique was used. All analytical steps were performed in three replicates with different concentrations of Cd, Cr, Pb, and Mn (19,20).

Statistical analysis
The differences between groups were determined using analysis of variance (ANOVA) considering significance level of 5%. Data were analyzed using SigmaStat 3.5 statistical software.

Results
Mean concentration of all metals (ppm) in the birds’ feathers sampled from three sites in Kano metropolis, 2019 are presented in Figure 2.

Figure 3 shows Pb concentration across the sampling sites for all samples. In site A, cattle egret had the highest concentration of Pb (89 ppm) while sunbird had the lowest one (56 ppm). Mallard drake had the highest concentrations of 78 and 74 ppm and sunbird had the lowest concentrations of 51 and 47 ppm in site B and C, respectively. No statistical differences were found between the sites in terms of the concentration of Pb among the bird species ($P>0.05$).

Figure 4 shows Cd concentration across the sampling sites for all samples. In site A, cattle egret had the highest concentration of Cd (45 ppm) while sunbird had the lowest one (20 ppm). In site B, hen had the highest concentration of Cd (41 ppm) while sunbird had the lowest one (23 ppm). In site C, guinea fowl had the highest concentration of Cd (41 ppm) while sunbird had the lowest one (21 ppm). No statistical differences were found between the sites in terms of the concentration of Cd among the bird species ($P>0.05$).

Figure 5 shows Mn concentration across the sampling sites for all samples. In site A, rooster had the highest concentration of Mn (0.024 ppm) while red winged starling had the lowest one (0.001 ppm). In site B, cattle egret had the highest concentration of Mn (0.022 ppm) while red winged starling had the lowest one (0.001 ppm). In site C, rooster had the highest concentration of Mn (0.019 ppm) while mallard drake had the lowest one (0.001 ppm). No statistical differences were found between the sites in terms of the concentration of Mn among the bird species ($P>0.05$).

Figure 6 shows Cr concentration across the sampling sites for all samples. Duck had the highest concentrations of 2.75, 2.67, and 2.55 ppm while tree sparrow had the lowest concentrations of 0.04, 0.02, and 0.02 ppm in sites A, B, and C, respectively. No statistical differences were found between the sites in terms of the concentration of Cr among the bird species ($P>0.05$).

Discussion
The present study demonstrated the concentration of some toxic metals in feathers of birds across various sites in Kano metropolis including industrial, commercial, and
residential areas as seen in Figure 1. Pb had the highest concentration followed by Cd, Cr, and Mn as presented in Figure 2. Similarly, Morrissey et al found out that among the metals detected in dipper feather samples, Pb had the highest concentration followed by Mn and Cd (21).

The highest concentration of metals in feathers was observed in site A (Sharada) followed by site B (Kurmi market) and site C (Rijiyar Lemo), however, the difference was not statistically significant ($P > 0.05$). Similarly, no significant difference was found between the concentration of metals among the bird species ($P > 0.05$). The differences in the concentration of Pb, Mn, Cd, and Cr between the three study sites could be attributed to different local contamination, pollution, or accumulation of metals in the diet of birds foraging in different localities. Site A is an industrial area which is characterized by discharge of pollutants into the environment and could contain a high burden of metals. These might be responsible for the overburden of Pb, Cr, Cd, and Mn in the birds’ feathers. However, site B and Care commercial and residential areas, respectively, which could have lower levels of pollutants compared to an industrial area (site A), hence, the lower concentrations of metals were recorded in these areas. Similarly, Eens et al has reported that the feathers of great tit nestlings contained significantly higher concentrations of Pb and lower concentrations of Zn in the polluted site compared to the reference site. However, there was no significant differences in the concentrations of As, Cd, and Cu between the polluted and reference sites ($P > 0.05$), which is consistent with the results of the present study (22). Cattle egret and mallard drake feathers contained the highest concentration of Pb while sunbird had the lowest one as seen in Figure 3. Jayakumar and Muralidharan reported high concentration of Cu ($53.31 \pm 23.19$ ppm) in little egret followed by cattle egret with concentration of $16.27 \pm 9.83$ ppm in liver (23). Hen and guinea fowl feathers recorded the highest concentration of Cd while sunbird revealed the lowest one as seen in Figure 4. The feathers of rooster contained the highest concentration of Mn while red wing starling had the lowest one as shown in Figure 5. Duck had the highest concentration of Cr while tree sparrow showed the lowest one as shown in Figure 6. Some other studies have revealed various degrees of metal contamination in the feathers of birds in polluted or non-polluted sites. Dauwe et al reported that great tit nestling (*Parus major*) from the site closest to the pollution source had significantly higher concentrations of Ag, As, Hg, and Pb in their feathers than those from the other sites (24). In addition, the concentration of metals in the feathers of nestlings were found to be significantly higher in the polluted site for most of the evaluated metals, which are comparable with those found in the earlier studies conducted along the same pollution gradient (25,26). The concentrations of Cd, Pb, and Cu in the feathers of great and blue tit were significantly higher in the polluted site than those in the reference site (22). Cd, Ni, Cu, Hg, and Se were evaluated in the birds’ feathers and their concentration exceeded the baseline concentrations found in the literature (27).

Many studies have been conducted on prey of birds and seabirds and have indicated higher accumulation of metals in feathers and other tissues (28-31). Several other studies have demonstrated accumulation of heavy metals in organs of bird species (32-34).
Heavy metals get into the keratinous structure of feathers when entered into the bloodstream during growth. Though, most metals could be deposited on the surface of the feathers in a quantity higher than the endogenous amounts of deposition (35). The heavy metals burden in the body of nestlings can be ascertained by the growing feathers. Interestingly, the accumulation of some essential trace elements could be regulated homeostatically to keep their concentrations in internal tissues physiologically balanced (36).

Contamination of feathers by exogenous heavy metals could be caused by their excretion from uropygial gland on feathers during preening or deposition from anthropogenic activities (37). Exogenous contamination has been identified as one of the major sources of heavy metals burden in birds’ feathers which could be an important path for accumulation of heavy metals in birds’ feathers (25,38,39). Such contamination can increase the concentration of most heavy metals in the birds’ feathers after their formation (40) and specifically to parts of the feathers that are exposed to the external environment. De Luca-Abbott et al stated that that diet had significantly affected the concentration of accumulated pollutants in Ardeids (41). Burger et al also reported that differences in metal concentrations between different sites could be due to differences in local exposure, atmospheric deposition, or foraging regimes of bird species (42). To support this, Dauwe et al found a positive correlation between feathers of great tit nestlings and caterpillars of lepidoptera acting as their prey for As and Pb (24).

Conclusion
The bird’s feathers contained various levels of heavy metals, among which Pb had the highest concentration followed by Cd, Cr and Mn. Feathers of cattle egret, mallard drake, hen, duck, rooster, and guinea fowl had the highest concentration of metals. The degree of fluctuations of the metals level reflects greatly the nature of the sites, whereby site A (Sharada industrial area) with a relative degree of pollution recorded the highest levels of metals. These results indicate slight metal contamination in the birds’ feathers especially in site A.

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Ethical issues
The authors certify that all data collected during the study are as stated in the manuscript, and no data from the study has been or will be published separately elsewhere. Ethical approval was obtained from Department of Biological Sciences, Bayero University, Kano, Nigeria (Ethical code: BUK/BS/VOL.1/00311).

Competing interests
The authors declare that they have no conflict of interests.

Authors’ contributions
The authors all contributed and involved actively in the problem analysis, experiments design and execution, data collection and analysis, manuscript preparation and approval.

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