

# An investigation of chromium toxicity in the wild population of black-headed oriole *Oriolus brachyrhynchus* (swainson, 1837) using atomic absorption spectrometry (AAS)

## Abstract

Chromium concentration in Black-headed oriole *Oriolus brachyrhynchus* was carried out using Atomic Absorption Spectrometry (AAS) to determine the bioaccumulation level in the feather, liver heart, skin, carcass, nestling feather and egg so as to say which of these parts carried more contaminant. A total of 30 birds were trapped using mist net and 150 of these parts were obtained. A total of 9 eggs and 9 nestling were also collected from different nest in the study areas for investigation. Descriptive statistics were used to express chromium concentration mg/kg in the selected parts. Chromium concentration in the different organs of the bird caught in the three sample sites are as follows: feather has the highest mean values of  $18.20 \pm 2.54$  mg/kg at Daudu, liver;  $6.25 \pm 2.38$  mg/kg at Daudu, heart;  $691 \pm 0.23$  mg/kg at Buruku, skin;  $4.43 \pm 2.0$  mg/kg at Daudu, carcass;  $28.49 \pm 2.68$  mg/kg at Buruku, egg;  $0.42 \pm 0.34$  mg/kg at Daudu and nestling feather;  $28.22 \pm 3.32$  mg/kg at Buruku. A significant correlation ( $r^2=0.824$ ) existed between the skin and the carcass at ( $P<0.01$ ) and also ( $r^2=0.668$ ) between feather and skin at ( $P<0.05$ ). Similar correlation of ( $r^2=0.721$ ) existed between feather and the carcass at ( $P<0.05$ ), while the weight of feather correlated ( $r^2=0.662$ ) with that of the heart at ( $P<0.05$ ). In addition, a negative correlation ( $r^2=-0.702$ ) existed between the weight of skin and that of the heart at ( $P<0.05$ ). Chromium concentration has the highest mean value of  $8.87 \pm 1.35$  mg/kg in maize at Daudu, and in rice;  $5.72 \pm 2.61$  mg/kg at Daudu, while guinea corn;  $6.92 \pm 1.39$  mg/kg also at Daudu. The concentration in soil and water was highest at Daudu with a mean of  $27.04$  mg/kg and  $0.61$  mg/l respectively. However, chromium concentration in the bird species in the study areas is still within permissible limit of WHO standard of  $50$  mg/kg, but far above WHO permissible level for crop  $1.30$  mg/kg and water  $0.1$  mg/l which calls for concern, as cereals are the major food consumed by the people in the study areas. This calls for continuous monitoring.

**Keywords:** chromium, bioaccumulation, black headed, AAS, toxicity

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Egwumah FA, Egwumah PO, Tyowua BT

Department of Wildlife and Range Management, University of Agriculture Makurdi, Nigeria

**Correspondence:** Egwumah FA, Department of Wildlife and Range Management, University of Agriculture Makurdi, Benue State, Nigeria, Tel +2347064621278, Email [egwumahattah@gmail.com](mailto:egwumahattah@gmail.com)

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**Abbreviations:** RCBD, randomized complete block design; AAS, atomic absorption spectrophotometer; NPK, nitrogen phosphorous potassium; WHO, world health organization

## Introduction

Chromium is commonly present in the surroundings as a result of its natural occurrence, human activities from industries and agriculture.<sup>1</sup> Most industrial processes produce metals as by-product in the ecosystem, and these may end-up in tissues of bird species existing in such natural ecosystems.<sup>2</sup> In most cases, the concentration may be higher than the permissible limit in avian species. Chromium (Cr) is a trace element that seems to be an essential micronutrient in animal diet. It occurs in two oxidation states such as trivalent ( $Cr^{3+}$ ) and hexavalent ( $Cr^{6+}$ ). However both are biologically active, but some differences in their metabolism are known.  $Cr^{6+}$  is more readily absorbed than  $Cr^{3+}$ , but  $Cr^{6+}$  toxicity is higher<sup>3</sup> in the environment. It occurs in rock strata rich in  $Cr^{3+}$  bearing minerals, in particular chromites are universally found in sedimentary rocks. Analysis of fertilizers marketed in Nigeria indicated that  $N_{15}P_{15}K_{15}$ ,  $N_{20}P_{10}K_{10}$  and  $N_{27}P_{13}K_{13}$  and phosphate fertilizers contained significant and varying amounts of chromium<sup>4</sup> as contaminants thereby, making it a potential

source of agro-contamination. Birds have been recognized as good indicators of environmental contamination because of their abundance, wide distribution, feeding at different trophic levels, and their long life span.<sup>1,5,6</sup> Several studies have shown that chromium in phosphate fertilizers can subsequently accumulate in soil and become readily available to plants and wild birds<sup>7,8</sup> that forage on such plants. Black headed oriole is a farmland dependent bird with a conservation status of least concern but with global changes, it is imperative to study a particular species living in a sensitive habitat such as agricultural ecosystem.<sup>6</sup> According to<sup>9</sup> chromium accumulation in soil does not depend on levels of phosphate fertilizer but on the levels of impurities in the phosphate rock used for the fertilizer production. In the study areas (Buruku, Daudu and Atega), the types of fertilizer commonly used are; Urea, Nitrogen-Phosphorous-Potassium (NPK), and Superphosphate.<sup>10</sup> Other anthropogenic inputs of chromium include a range of industrial activities, alloys, chrome plating, dyes and pigments, textiles, leather tanning, and wood preserving.<sup>11</sup> These anthropogenic activities have led to widespread environmental chromium contamination. The main objective of the study is to determine, whether the chromium concentration in the various parts of the birds differs with location.

## Materials and methods

### Study area

The study sites were located at Buruku on Latitude 7°27'35.6"N and Longitude 9°12'20.5"E in Buruku LGA, Daudu on Latitude 7°55'53.0"N and Longitude 8°34'53.9"E in Guma LGA and Adegga on Latitude 7°01'47.4"N and Longitude 8°15'28.0"E in Obi LGA of Benue State.

### Sampling technique

Using selective sampling technique which is a non-probability sampling techniques based on personal choice without statistical bias. The study sites are not a conservation area and the local people are predominantly peasant farmers. A total of 28 hectares of farmland area was selected in each of these study sites. The 28 ha sampling plot was further divided into four sampling units of 7ha, where bird capturing activities took place, but earlier ethical permit was obtained from the Department of Forest Protection and Management, Ministry of Water Resources and Environment, Benue State, Nigeria. A mist net was set in each sampling unit and the net was checked after every 2-3hours for a catch. Three replicates of nestling feathers and eggs were collected and stored in plastic sampling bottles from the tree species used as breeding ground by birds. Ten birds were trapped at the various locations. The birds were slaughtered and weighed before they were defeathered, the feathers were dried in an oven and kept in polyethene bags and sealed tightly. The defeathered birds are dissected to remove the skin, heart and liver which were kept in a polyethene bags and stored in a dessicator for chromium analysis. Three replicates of Rice, maize and guinea corn were also collected from different farmland as component of biological pathway for contamination in some wild birds. Three replicates of water and soil samples were equally collected from the study areas for chromium concentration investigation.

### Sample cleaning

The feather samples collected were cut into about 0.3cm using a stainless steel scissors and first rinsed in ethanol, then washed three times in distilled water and then finally washed again in ethanol in accordance to the recommendation of International Atomic Energy Agency.<sup>12</sup> These were placed in crucibles and dried in an oven at 75°C±5°C for 25 minutes. About 0.2mg of treated feather sample was weighed and stored in an inert plastic container of 10cm<sup>3</sup> capacity, corked tightly and kept for chromium analysis using a Flame Atomic Absorption Spectrometry. The skin, heart, liver and the carcass were dried in an oven equipped with circulation system at 60°C for 48hr, and homogenized using a porcelain mortar. Approximately 0.2mg of dry sample was treated with 7ml of concentrated nitric acid and heated for 20min in the microwave oven, as described by Edison.<sup>13</sup> The resultant solution was transferred into a 100ml volumetric flask and made up to volume with distil water. The solution was stored at 4°C in polyethylene bottles until chromium was analyzed, using a Flame Atomic Absorption Spectrometry. The same procedure was followed for rice, maize, guinea corn, water and soil collected and digested for this study.

### Quality control

To ensure accuracy of results, two blanks were prepared for the avian parts, cereal and water namely; A and B Blank A comprises of 7ml of concentrated nitric acid and 2ml of hydrogen peroxide which were heated for 30 minutes and the content was allowed to cool down

to a room temperature then transferred into a volumetric flask and diluted to a final volume of 100ml with distil water whereas, blank B was 100ml of distil water only. The contaminants present in blank A and B were subtracted from the contaminants present in parts of the birds, crops and water collected and digested for this study to obtain the actual concentration of contaminants present. Similarly, two blanks were also prepared for soil samples namely C and D. Blank C comprises of 7ml of freshly prepared aqua-regia (3ml HNO<sub>3</sub>+9ml HCl) in the ratio 1:3 which was heated for 45 minutes and the content is allowed to cool down to a room temperature then transferred into a volumetric flask and diluted to a final volume of 100ml with distil water whereas, blank D was 100ml of distil water only. The contaminants present in blank C and D were subtracted from the contaminants present in all the soil samples collected and digested for this study to get the actual concentration of contaminants in soil. In addition to further ensure accuracy, the AAS machine was calibrated using a known concentration of metals. These metals were run in the AAS machine to determine the absorbance and concentration. Calibration curves were obtained by plotting concentration against absorbance.

### Experimental design and data analysis

Using Randomized Complete Block Design (RCBD) the data was analyzed, where locations represented the blocks and the bird species the treatment. Pearson correlation was used to test the correlation between weight of parts and chromium concentrations in parts ( $P<0.05$ ). The Summary statistics of chromium concentration (mg/kg) in selected parts of Black-headed orioles was expressed in mean values with their respective standard deviation, minimum and maximum values and results were presented in tables and bar chart.

## Result

A total of 168 organs were obtained from Black-headed orioles for chromium concentration investigation. Table 1 shows the Summary statistics of chromium concentration in selected organs of Black-headed orioles expressed in mean values with their respective standard deviation, minimum and maximum values. Chromium concentration in feathers ranged from 7.19-13.83mg/kg in birds trapped from Buruku with a mean of 10.35±2.36 mg/kg whereas chromium concentration in birds trapped from Daudu ranged from 14.94-22.13mg/kg with a mean of 18.20±2.54mg/kg and birds trapped from Adegga ranged from 4.23-9.96mg/kg with a mean of 7.94±2.29mg/kg. However, Daudu has the highest chromium concentration of 18.20±2.54mg/kg in feathers of the birds. Chromium concentration in liver of the birds trapped from Buruku ranged from 3.22-9.41mg/kg with a mean of 6.04±2.28mg/kg whereas the concentration in liver of birds trapped from Daudu ranged from 3.50-9.70mg/kg with a mean of 6.26±2.38mg/kg but the concentration in liver of birds from Adegga ranged between 3.15-8.84mg/kg with a mean of 5.29±2.18mg/kg. However, birds from Buruku have the highest chromium concentration in the liver of the birds in the study areas.

Chromium concentration in hearts of the birds trapped from Buruku ranged from 0.28-0.94mg/kg with a mean of 6.91±0.23mg/kg whereas the concentration in heart of birds trapped from Daudu ranged from 0.30-0.96mg/kg with a mean of 0.70±0.22mg/kg but the concentration in heart of birds trapped from Adegga ranged from 0.10-0.76mg/kg with a mean of 2.76±0.21mg/kg. Buruku has the highest chromium concentration in the heart of the bird species. The concentration in skin of the birds trapped from Buruku ranged from 2.24-8.85mg/kg with a mean of 4.21±2.00mg/kg. Whereas, the concentration in skin of birds trapped from Daudu ranged from 2.30-

9.03mg/kg with a mean of 4.43±2.00mg/kg making it the highest chromium concentration in the skin of the birds in the study areas, but chromium concentration in skin of birds trapped from Adega ranged from 1.00-1.39mg/kg with a mean of 1.29±0.14mg/kg. The concentration in carcass of the birds trapped from Buruku after the vital organs were removed ranged from 24.90-32.09mg/kg with a

mean of 28.49±2.68mg/kg whereas chromium concentration in the carcass of birds trapped from Daudu ranged from 20.47-27.67mg/kg with a mean of 23.96±2.69mg/kg but the concentration in carcass of birds trapped from Adega ranged from 10.51-21.58mg/kg with a mean of 15.51±4.17mg/kg. Therefore, Buruku has the highest chromium concentration in the carcass of the study species.

**Table 1** Summary statistics of chromium concentration (mg/kg) in selected organs of Black-headed orioles trapped from Benue State, Nigeria

Location and tissues of black-headed orioles	N	Mean±SD	Minimum	Maximum
<b>Buruku</b>				
Feathers	10	10.35±2.36	7.19	13.83
Liver	10	6.04±2.28	3.22	9.41
Heart	10	6.91±0.23	0.28	0.94
Skin	10	4.21±2.00	2.24	8.85
Carcass	10	28.49±2.68	24.9	32.09
Eggs	3	0.36±0.08	0.28	0.43
Nestling feathers	3	28.22±3.32	24.9	31.54
<b>Daudu</b>				
Feathers	10	18.20±2.54	14.94	22.13
Liver	10	6.26±2.38	3.5	9.7
Heart	10	0.70±0.22	0.3	0.96
Skin	10	4.43±2.00	2.3	9.03
Carcass	10	23.96±2.69	20.47	27.67
Eggs	3	0.42±0.34	0.39	0.45
Nestling feathers	3	22.68±3.37	20.47	26.56
<b>Adega</b>				
Feathers	10	7.94±2.29	4.23	9.96
Liver	10	5.29±2.18	3.15	8.84
Heart	10	2.76±0.21	0.1	0.76
Skin	10	1.29±0.14	1	1.39
Carcass	10	15.51±4.17	10.51	21.58
Eggs	3	0.05±0.01	0.04	0.06
Nestling feathers	3	6.62±2.96	4.33	9.96

Chromium concentration in eggs of the birds collected from Buruku ranged from 0.28-0.43mg/kg with a mean 0.36±0.08mg/kg whereas chromium concentration in eggs of birds collected from Daudu ranged from 0.39-0.45mg/kg with a mean of 0.42±0.34mg/kg making it highest chromium concentration in eggs of the birds from the study areas, but chromium concentration in eggs of birds collected from Adega ranged from 0.04-0.06mg/kg with a mean of 0.05±0.01mg/kg. Chromium concentration (mg/kg) in nestling feathers of the birds trapped from Buruku ranged from 24.90-31.54mg/kg with a mean 28.22±3.32mg/kg whereas chromium concentration in nestling feather of birds from Daudu ranged from 20.47-26.57mg/kg with a mean of 22.68±3.37mg/kg but the concentration of chromium in the nestling feather of birds from Adega ranged from 4.33-9.96 with a mean of 6.62±2.96mg/kg. However, Buruku has the highest

chromium concentration in the nestling feathers of bird species in the study areas (Table 1).

Metal levels are in parts per million (ppm) and are presented as wet weight for eggs and dry weight for other organs. However, Table 2 shows that, very high positive significant correlation ( $r^2=0.824$ ) existed between chromium concentration in the skin and chromium concentration in the carcass ( $P<0.01$ ) whereas very high significant positive correlation ( $r^2=0.668$ ) also existed between chromium concentration in the feather and chromium concentration in the skin ( $P<0.05$ ). There was also similar correlation ( $r^2=0.721$ ) between chromium concentration in the feather and chromium concentration in the carcass ( $P<0.05$ ). Another correlation ( $r^2=0.662$ ) also existed between weight of feather and weight of heart ( $P<0.05$ ). Finally a very

high negative correlation ( $r^2=-0.702$ ) also existed between weight of skin and weight of heart ( $P<0.05$ ) (Table 2).

\* = ( $P < 0.05$ )

\*\* = ( $P < 0.01$ )

Table 3 from the analysis, a total of 27 crop samples were collected from Buruku, Daudu and Adega in Benue State for chromium concentration investigation and chromium concentration in maize harvested directly from farmland in Buruku ranged from 7.19-9.96mg/kg with a mean  $8.11\pm1.59$ mg/kg whereas chromium concentration in maize harvested from Daudu ranged from 7.75-10.37mg/kg with a mean of  $8.87\pm1.35$ mg/kg, but chromium concentration in maize harvested from Adega ranged from 1.26-3.25 with a mean of  $2.17\pm1.01$ mg/kg using atomic absorption spectrophotometer (AAS). However, maize collected from Daudu had the highest chromium concentration from the study areas. Chromium concentration in rice

harvested from farmland in Buruku ranged from 2.75-6.61mg/kg with a mean  $5.14\pm2.09$ mg/kg whereas chromium concentration in rice harvested from Daudu ranged from 2.77-7.75 mg/kg with a mean of  $5.72\pm2.61$ mg/kg, but chromium concentration in rice harvested from Adega ranged from 1.56-2.70mg/kg with a mean of  $2.09\pm0.57$ mg/kg using atomic absorption spectrophotometer (AAS). Therefore rice harvested from Daudu had highest chromium concentration from the study areas. Chromium concentration in guinea corn harvested directly from farmland in Buruku ranged from 5.10-7.25mg/kg with a mean  $6.13\pm1.08$ mg/kg whereas the concentration in guinea corn harvested from Daudu ranged from 5.50-8.27mg/kg with a mean of  $6.92\pm1.39$ mg/kg, making it the highest chromium concentration in the guinea corn from the study areas but chromium concentration in guinea corn harvested from Adega ranged from 1.50-2.30mg/kg with a mean of  $1.83\pm0.42$ mg/kg using atomic absorption spectrophotometer (AAS) (Table 3).

**Table 2** Correlation between chromium concentrations in different organs and wet weight of different organs

	WF	WC	WL	WS	WH	CrCF	CrCC	CrCL	CrCS	CrCH
WF	1									
WC	0.54	1								
WL	-0.272	-0.104	1							
WS	-0.151	-0.348	-0.593	1						
WH	0.662*	0.438	0.201	-0.702*	1					
CrCF	-0.086	0.046	-0.022	-0.458	0.298	1				
CrCC	-0.52	-0.376	-0.04	0.057	-0.213	0.721*	1			
CrCL	-0.26	0.133	0.338	0.177	-0.417	-0.335	-0.016	1		
CrCS	-0.288	-0.223	0.013	-0.054	-0.072	0.668*	0.824**	-0.006	1	
CrCH	-0.452	-0.012	0.124	-0.299	0.159	0.605	0.63	-0.138	0.651*	1

WF: Weight of feather CrCF: Chromium concentration in feather

WL: Weight of liver CrCL: Chromium concentration in liver

WC: Weight of carcass CrCC: Chromium concentration in carcass

WS: Weight of skin CrCS: Chromium concentration in skin

WH: Weight of heart CrCH= Chromium concentration in heart

**Table 3** Summary statistics of Chromium concentration (mg/kg) in crops harvested from the study locations

Locations	N	Mean±SD	Minimum	Maximum
<b>Buruku</b>				
Maize <i>Zea mays</i>	3	$8.11\pm1.59$	7.19	9.96
Rice <i>Oryza sativa</i>	3	$5.14\pm2.09$	2.75	6.61
Guinea corn <i>Sorghum bicolor</i>	3	$6.13\pm1.08$	5.1	7.25
<b>Daudu</b>				
Maize <i>Zea mays</i>	3	$8.87\pm1.35$	7.75	10.37
Rice <i>Oryza sativa</i>	3	$5.72\pm2.61$	2.77	7.75
Guinea corn <i>Sorghum bicolor</i>	3	$6.92\pm1.39$	5.5	8.27
<b>Adega</b>				
Maize <i>Zea mays</i>	3	$2.17\pm1.01$	1.26	3.25
Rice <i>Oryza sativa</i>	3	$2.09\pm0.57$	1.56	2.7
Guinea corn <i>Sorghum bicolor</i>	3	$1.83\pm0.42$	1.5	2.3

A total of nine (9) soil samples and nine (9) water samples were collected from different location in Benue state for chromium concentration investigation. Apart from that, soil samples from Buruku ranged from 24.9-26.56mg/kg whereas Daudu has chromium concentration that ranged from 25.01-28.32mg/kg and samples from Adega ranged from 2.31- 4.98mg/kg (Figure. 1). However, soil sample from Daudu had the highest chromium concentration of 28.32mg/kg in soil. Water samples from Buruku range from 0.40-0.63mg/l in chromium concentration, whereas chromium concentration in water samples collected from Daudu ranged from 0.50-0.70mg/l. Water samples from Adega ranged from 0.20-0.27mg/l (Figure 2). Therefore water samples from Daudu had the highest chromium concentration of 0.70mg/l (Figures 1& 2).

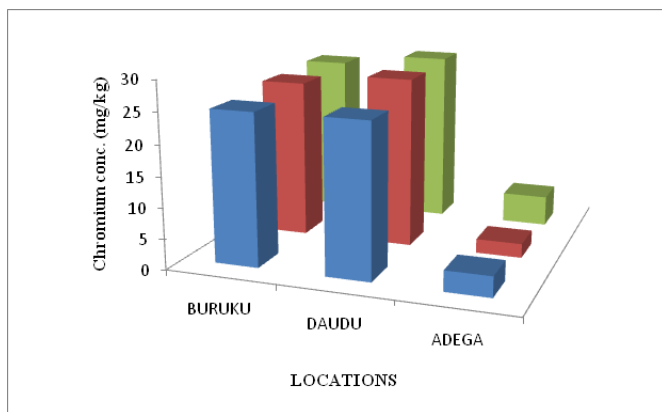


Figure 1 Chromium concentrations in soil samples.

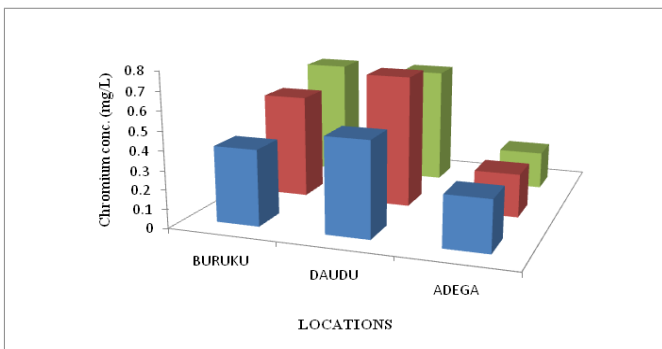


Figure 2 Chromium concentrations in water samples.

## Discussion

The high concentration of chromium in carcass as compared to other organs was due to residual accumulation. In as much as the concentration differs in the organs, environment is the fundamental determinant of its accumulation in living organism. Therefore in terms of magnitude of chromium concentration in organism in the same locality, this current research work recorded a concentration of 28.49±2.68mg/kg in carcass of birds which is lower than the research work of<sup>14</sup> who reported concentration of 92.9mg/kg and 88.5mg/kg in *Tilapia zilli* and *Clarias gariepinus* from River Benue. The reasons for these differences in chromium concentration could be due to differences in species, habitat, nature of anthropogenic activities discharging into the river and differences in rate of clearance in the living organisms. This work also recorded a higher chromium concentration compared to the research work of<sup>15</sup> that reported a lower value of 16.29±0.133mg/kg in excreta of Cattle Egret in India. We equally recorded a lower chromium concentration compared to

the research work of<sup>16</sup> who reported a value of 301.28mg/kg in shell of mollusk and 389.30mg/kg in the blood of Red winged black bird in New York, USA. In addition<sup>17</sup> reported a lower concentration of 0.69mg/kg in insect. The reasons for the varied concentration recorded in this current work and other research work are differences in species, trophic level, environment, methods of metal determination and the nature of the anthropogenic processes in the studied areas. In a similar manner, this work is also at variance with the work of<sup>17</sup> who reported a lower chromium concentration of 0.23±0.03mg/kg and 0.18±0.02mg/kg in the liver of House Sparrows from urban and rural habitats in southern Finland. This recent work is not in agreement with the research work of<sup>18</sup> who reported a higher chromium concentration levels in the shorebirds such as Red knot, Sanderling and Semipalmated sandpiper with a chromium concentration of 578±83mg/kg, 764±260mg/kg and 1149±294mg/kg respectively in Delaware Bay in New Jersey, USA and the reasons could be differences in species, location and methods of contaminant analysis. From this research finding, the concentration of chromium in all the organs of the said species was below threshold of 50mg/kg as recommended by WHO.<sup>19</sup> However, in larger quantities and in different forms, chromium can be toxic and carcinogenic.<sup>15</sup> It was reported that chromium concentration in mallard duck produced adverse effects in embryo development, viability and hatching.<sup>15</sup>

Chromium is necessary in organisms at certain amounts but becomes harmful when in excess or above the required threshold level of 50mg/kg in wild birds as recommended by WHO.<sup>19</sup> The effects of chromium depends on the quantity accumulated, organs affected, bird species, and environmental factors. From this research findings chromium concentration has not exceeded the permissible limit in birds as recommended by World Health Organization, therefore there is no call for concern in the study areas. This current research work recorded a lower chromium concentration in water compared to the report of<sup>20</sup> who reported chromium concentration that range from 1.31 to 2.89mg/l in water. Similarly, this current work recorded a higher chromium concentration in soil compared to the aforementioned author. The reasons for the varied concentration recorded in this current research work and other work are differences in environment, methods of metal determination and the nature of the anthropogenic processes in the studied areas. The maximum permissible limit for chromium in water is 0.1mg/l<sup>20</sup> and the permissible limit of chromium for plants is also 1.30mg/kg as recommended by World Health Organization<sup>20</sup> and from our research findings, the WHO permissible limit has been exceeded because this present work recorded a higher chromium concentration in both water and crop plants which are the major biological pathway of avian contamination. Although, this present work has not exceeded the permissible limit of 1- 1000mg/kg for chromium content in the soil. Higher concentration in food chain can easily make up the deficiency in chromium and may also become a threat to birds in the nearest future.

## Conclusion

The reason for low chromium concentrations in the species from this study is due to its role in metabolic improvement in bird species. However this does not call for concern since the permissible limit for wild birds is not exceeded. The possible causes of chromium concentrations in water and crops may be attributed to increase the use of chemical fertilizers on farm because Urea, Nitrogen-Phosphorous-Potassium (NPK), and Superphosphate contain chromium that can contaminate the crops and water. However, continuous monitoring is necessary.

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## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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