

## Heavy metal concentration in some commercial poultry feeds available in Abuja, Nigeria

Noela Chinyelu Igwemmar\*, Samuel Esimikwame Kakulu and Mary Sunday Dauda

*Department of Chemistry, University of Abuja, P. M. B. 117, Gwagwalada - Abuja, Nigeria.*

World Journal of Advanced Research and Reviews, 2022, 16(02), 005–015

Publication history: Received on 20 September 2022; revised on 27 October 2022; accepted on 29 October 2022

Article DOI: <https://doi.org/10.30574/wjarr.2022.16.2.1110>

### Abstract

The concentrations of heavy metals in some commercial poultry feeds available in Abuja, Nigeria was investigated. Poultry feed rations (starter, grower, finisher and layer) of seven different feed brands available in Abuja were purchased from their various distribution outlets. The nitric acid digestion method followed by Flame Atomic Absorption Spectrophotometry technique were used for metal content quantification. Analytical method validation was performed using IAEA-V-8 Rye flour certified reference material and good recoveries were obtained in all the metals indicating the accuracy of the applied analytical procedure. The mean of heavy metal concentrations ( $\mu\text{g/g}$  dry weight) in the poultry feeds were  $2.31 \pm 1.23$ ,  $0.43 \pm 0.20$ ,  $1.66 \pm 0.82$ ,  $23.35 \pm 5.26$ ,  $238.09 \pm 45.80$ ,  $0.61 \pm 0.26$ ,  $99.57 \pm 15.63$  and  $68.21 \pm 11.92$  for Pb, Cd, Ni, Cu, Fe, Co, Mn and Zn respectively. Generally, the levels of iron, manganese, zinc and copper in the poultry feeds were higher than FAO/WHO, National Research Council (NRC) and Standard Organisation of Nigeria (SON) maximum recommended limits in feeds. Pearson correlation analysis on the metals in poultry feeds showed a significant ( $p < 0.01$ ) positive strong correlations between Co/Ni ( $r = 0.658$ ), Mn/Cu ( $r = 0.566$ ), Zn/Cu ( $r = 0.683$ ) and Zn/Mn ( $r = 0.758$ ) suggesting that these metals could have had the same pathway into the feeds.

**Keywords:** Certified Reference Material; Heavy metals; Nitric acid; Poultry feed; Rye flour; Standard Organization of Nigeria

### 1. Introduction

Pollution of the environment with heavy metals is a major global problem posing serious health risk to livestock and humans. Heavy metals in the Nigerian environments is currently an area of great interest due to the ubiquity of exposure. Industrialization, urban human activities and improvement in agricultural chemicalization has contributed to the increase of heavy metals in the environment. Heavy metals are group of metals and metalloids that are associated with contamination and potential toxicity or ecotoxicity [1], having densities higher than  $5 \text{ g/cm}^3$ , or at least 5 times greater than the density of water [2,3]. Some heavy metals such as arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) are toxic or poisonous even at low concentrations and have no known biological roles in living organisms [4,5] while others such as iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) are essential micronutrients but can become toxic at higher concentrations than required for normal growth [6].

Poultry is a category of bird raised for meat and egg production or hunted for economic value e.g. chickens, turkeys, guinea fowls, pigeons, ducks and geese, quails, pheasants, ostriches, pigeons and doves [7]. Poultry feed is any single and composite material whether processed, semi-processed or raw, intended to be fed directly to poultry [8]. Feeds for poultry are formulated to contain protein, carbohydrate, vitamins, minerals and trace metals necessary for proper growth, egg production and bird health, hence, they are referred to as complete feed. For development and growth rate

\* Corresponding author: Noela Chinyelu Igwemmar  
Department of Chemistry, University of Abuja, P. M. B. 117, Gwagwalada - Abuja, Nigeria.

improvement in poultry, different heavy metals are added in poultry feeds. However, excessive use of these metals in poultry feeds can result in deleterious effect on poultry and human being on its consumption [9].

Feed ingredients and poultry feed can be contaminated with undesirable substances (heavy metals, pesticides) which may be found in the environment and/or the production process [10]. Livestock that consumes metal contaminated feeds can bioaccumulate the contaminants in their tissues over time since heavy metals in their elemental state cannot be metabolized or destroyed [11-14]. Given the prevalence of heavy metals in the environment, contamination of animal feeds by these metals is inevitable, hence there is need to minimize the contamination thereby reducing the effects on animal and human health [15]. Worldwide food safety is a major public concern because of the risks associated with exposure to heavy metals.

Lead is a toxic metal with no known nutritional value. Toxic effects of lead are observed on the haematopoietic, nervous, gastrointestinal and renal systems [16]. Cadmium is one of the most important environmental and industrial toxicant that affects the reproductive tracts, bone metabolism, brain, heart and blood vessel, kidney and lungs [17,18]. Nickel is a controversial element. Some believe it to be an essential element [19], but others class it as an element of common environmental concern because it is highly toxic and carcinogenic [20]. Nickel has been linked to chronic side effects such rhinitis, sinusitis, perforations of the nasal septum, and asthma.

Copper is a vital dietary nutrient, although only small amount of the metal is needed for well-being [21]. Acute poisoning due to copper ingestion is characterized by vomiting, lethargy, acute haemolytic anaemia, renal and liver damage, neurotoxicity, increased blood pressure and respiratory rates. Iron is an essential trace metal and the fourth most abundant element in the earth's crust [22]. Iron toxicity has been linked to arthritis, anorexia, fatigue, gut damage, increased oxidative stress, cancer and heart disease, hyperactivity, headaches, liver damage, metabolic acidosis.

Cobalt is an essential trace mineral for humans, animals and plants. Occupational exposure of human to cobalt have reported primarily respiratory effects, including decreased pulmonary function, asthma, pneumonia, interstitial lung disease, wheezing and dyspnea. Deficiency of cobalt in birds, results in slow growth, mortality, reduced hatchability [23]. Manganese is a trace mineral that is both nutritionally essential and potentially toxic [24]. Manganese deficiency in man has been associated weak bones (osteoporosis), anemia, chronic fatigue syndrome and low immunity. Deficiency in chickens can result in perosis, poor hatchability, enlargement of joints and staggering gait [23]. Zinc is an essential micro-nutrient that performs important biochemical functions and it is necessary for maintaining health throughout life [25]. Acute zinc poisoning manifestations include nausea, cramps, vomiting, diarrhoea and fever.

Several studies on heavy metal in poultry feeds have been reported by different authors. For example, Mohammad et al. [26] in their study assessed the quality of the poultry feed and its effect in poultry products in Bangladesh. Zhang et al. [27] examined the content of heavy metals in animal feeds and manures from farms of different scales in northeast China while Okoye et al. [28] measured the heavy metals levels in chicken feeds sold in south-eastern, Nigeria. A survey on transfer of heavy metal contaminants from animal feed to animal products was reported by Makridis et al. [29]. In Awka metropolis, Okeke et al. [30] assessed the heavy metal levels in feeds and litters of chickens. Also, Wang et al. [31] in their study in China, evaluated the changes in heavy metal contents in animal feeds and manures in an intensive animal production region. This study is therefore undertaken to assess the heavy metal contents of commercial poultry feeds available in Abuja, Nigeria since no similar study have been conducted in this area.

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## 2. Material and methods

### 2.1. Study Area

Abuja, the capital city of Nigeria is situated in the centre of the country within the Federal Capital Territory (FCT). The geographic coordinates of Abuja are latitude 9°03'28" N and longitude 7°29'42" E. The capital territory is currently made up of six Area Councils, comprising Abaji, Abuja Municipal, Gwagwalada, Kuje, Bwari and Kwali [32].

### 2.2. Sample Collection

Seven commercial poultry feed brands designated as Feeds A, G, H, L, S, T and V in their various rations (starter, grower, finisher and layer) were purchased on each sampling day from their distributor outlets in Abuja, Nigeria. Feed samples were collected twice a month from October 2021 to December 2021. For each ration (starter, grower, finisher and layer) of every feed brand, samples were collected from ten (10) different bags (having the same production batch number) containing the same feed ration. The feed samples were transported in ziploc bags to the laboratory.

### 2.3. Sample Pretreatment

Same type of each feed ration from the ten (10) different bags were mixed together to form one composite sample. This was done for all the feed brands. The feed samples were ground using a micro grinder (A-10 Analytical mill Tekmar, Germany), and passed through 0.25 mm mesh sieve to obtain a uniform particle size. They were labelled accordingly and stored in pre-washed air tight sample containers at ambient temperature until ready for analysis.

### 2.4. Treatment of Glassware and Sample containers

Glassware and sample containers were washed with a detergent solution, rinsed many times with tap water and soaked overnight in 10 % (v/v) nitric acid for 24 hours. The containers and glasswares were later rinsed severally with deionized water. They were dried properly before use.

### 2.5. Reagents

All reagents used in this study were of analytical grades. Nitric acid (65% HNO<sub>3</sub>) was manufactured by Loba Chemie Pvt. Ltd., Mumbai, India. Standard stock solution (1000 mg/l) of Pb, Cd, Ni, Cu Fe, Co, Mn, and Zn were products of Sigma Aldrich, UK. A certified reference material (CRM), IAEA-V-8 Rye Flour was obtained from International Atomic Energy Agency (IAEA Vienna, Austria). Deionized water was used throughout the study.

### 2.6. Validation of Digestion Method

To establish the validity and reliability of the analytical procedure a certified reference material (CRM), IAEA-V-8 Rye flour was used. The reference material was chosen as it was the closest available to samples under investigation (organic matrix). Prior to the analysis, the reference material was dried to a constant weight at 105 °C as indicated in the certificate. Analytical method validation was conducted using the conventional acid digestion method described by Mohammed et al. [33] with a little modification. Two gram (2.0 g) of the CRM, Rye flour was weighed into a 250 ml conical flask and 20 ml of concentrated 65% HNO<sub>3</sub> was added into it. The mixture was digested on a hot plate (100 °C) in the fume hood for about 2 h until no brown fumes were given off and digestion is complete. The digest was allowed to cool and filtered through Whatman grade 41 filter paper into a 100 ml volumetric flask and the filtrate made up to mark with deionized water. The solution was transferred into sample container and kept in a refrigerator at 4 °C until analysis. Analysis was carried out in triplicate. Blank digestion was performed in parallel with the CRM sample keeping all digestion steps the same so as to ascertain the contribution of reagents to the metal levels. The percentage recovery was calculated using the formula:

$$\% \text{ Recovery} = \frac{\text{measured concentration(mg/kg)}}{\text{certified value(mg/kg)}} \times 100$$

#### 2.6.1. Sample Digestion

The CRM analysis result showed good recoveries for all the metals indicating the efficiency of the digestion method. Hence, the digestion method described by Mohammed et al. [33] was adopted in the digestion of the poultry feeds. Two gram (2.0 g) of each dried poultry feed sample was used for the digestion. All the digestion steps applied in the CRM analysis was replicated in the poultry feed digestion. Analysis was carried out in triplicate. Blanks were prepared by taking appropriate reagents through the same procedure as the feed samples.

### 2.7. Preparation of standard solution

Working standard solutions were prepared by serially diluting appropriate aliquot of the standard stock (1000 mg/l) solution of Pb, Cd, Ni, Cu Fe, Co, Mn, and Zn. All the working standard solutions were prepared fresh daily. Calibration blanks were also prepared for calibration of analytical instrument.

### 2.8. Metal Determination

The concentrations of heavy metals in the sample solutions were determined using Flame Atomic Absorption Spectrophotometer (FAAS) (Thermo Scientific iCE 3000 series, UK). The determinations were carried out according to the instrument working conditions for analysis. Air-acetylene flame was used for the determination of each metal. The wavelength (nm) of the metals under study were Pb (217.0), Cd (228.8), Ni (232.0), Cu (324.8), Fe (248.3), Co (240.7), Mn (279.5) and Zn (213.9).

## 2.9. Statistical Analysis

The results were subjected to one-way analysis of variance (ANOVA) to determine any statistically significant difference in the concentration of heavy metals in the poultry feed samples followed by Duncan's multiple range post hoc test to identify the pairs that differ. Pearson correlation analysis was performed to investigate correlations between heavy metal concentrations in feeds. Differences were considered significant at p-values  $< 0.01$  and  $< 0.05$ . All statistical analysis was performed using Statistical Package for Social Science (SPSS) version 23.0

## 3. Results

The results of the certified reference material (CRM) IAEA-V-8 Rye flour analysis is shown in Table 1. The percentage recovery of heavy metals in the CRM ranged from 94.12 % to 117.39 %. This revealed a good agreement between measured and certified concentrations indicating that the analytical method is well suited for heavy metal determination in the CRM sample and possibly similar matrices. Hence, the analytical procedure was adopted in this study.

Table 2 presents the concentrations of heavy metals in different poultry feed brands. The highest concentration of lead was found in Feed L brand while it was not detected in Feeds S, T and V respectively. Generally, the levels of lead in the feed brands are in the order: Feed L > Feed G > Feed A > Feed H. The highest cadmium concentration was observed in Feed S brand while Feed H brand had the least concentration. The levels of cadmium in Feed A, Feed L, Feed S, Feed T and Feed V were all higher than the mean cadmium concentration (Table 2). The concentrations of nickel in the feed brands are in the order of Feed S > Feed A > Feed H > Feed G > Feed L > Feed T > Feed V. The highest concentration of nickel ( $2.77 \pm 0.55 \mu\text{g/g}$  dry weight) was detected in Feed S brand while Feed V gave the lowest concentration in nickel. For copper concentration in the brands, the lowest value was found in Feed H brand while the Feed T brand gave the highest concentration. Also, the levels of Cu in the feed brands are in the order: Feed T > Feed V > Feed G > Feed A > Feed S > Feed L > Feed H (Table 2).

**Table 1** Result of metal content in IAEA-V-8, Certified Reference Material (CRM)

Metal	Certified [or information] value <sup>a</sup> ( $\mu\text{g/g}$ )	This Study <sup>b</sup> ( $\mu\text{g/g}$ )	% Recovery
Pb	NA <sup>c</sup>	-	-
Cd	$0.017 \pm 0.01$	$0.016 \pm 0.004$	94.12
Ni	NA	-	-
Cu	$0.95 \pm 0.19$	$0.93 \pm 0.03$	97.89
Fe	$4.1 \pm 0.7$	$3.99 \pm 0.05$	97.32
Co	$0.0023 \pm 0.0009$	$0.0027 \pm 0.004$	117.39
Mn	$2.06 \pm 0.12$	$1.99 \pm 0.06$	96.60
Zn	$2.53 \pm 0.33$	$2.49 \pm 0.33$	98.84

a. IAEA- V-8, Rye flour CRM: certified [or information] value (Mean  $\pm$  SD) b. This study - Mean  $\pm$  SD (n = 2) c. Not available

The Feed H brand gave the highest value of iron while Feed G brand has the lowest value. Some feed brands: Feed H, Feed S, Feed A and Feed V recorded iron concentrations higher than the mean iron concentration. Cobalt concentration was observed to be highest in Feed A brand ( $0.98 \pm 0.24 \mu\text{g/g}$  dry weight) while the lowest was found in Feed T brand ( $0.31 \pm 0.00 \mu\text{g/g}$  dry weight) (Table 2). Generally, the levels of cobalt in the feed brands are in the order of Feed A > Feed S > Feed L > Feed V > Feed H > Feed T. Manganese concentration was lowest in Feed H brand while the Feed T brand gave the highest concentration. The concentrations of manganese in Feed T, Feed L, Feed G and Feed S brands were all higher than the mean manganese concentration. The Feed T brand gave the highest zinc concentration while the Feed H brand gave the lowest concentration. The levels of zinc in the feed brands are in the order of Feed T > Feed G > Feed L > Feed V > Feed S > Feed A > Feed H. Tables 3 – 6 shows the concentrations ( $\mu\text{g/g}$  dry weight) of heavy metals in the different poultry feeds rations.

**Table 2** Concentration ( $\mu\text{g/g}$  dry weight) of heavy metals in the different feed brands

Feed brands	Metals							
	Pb	Cd	Ni	Cu	Fe	Co	Mn	Zn
Feed A	1.56±1.82	0.53±0.12	2.35±0.84	25.42±8.76	270.95±64.82	0.98±0.24	96.94±6.45	63.47±10.33
Feed G	2.39±0.06	0.20±0.27	1.81±1.33	25.76±10.29	164.81±67.28	ND	103.69±52.73	80.72±25.54
Feed H	1.28±0.00	0.09±0.00	1.82±0.71	15.58±5.08	283.09±60.99	0.32±0.17	81.00±5.80	49.75±13.31
Feed L	4.02±3.66	0.51±0.27	1.66±0.57	18.56±4.55	229.78±57.11	0.71±0.00	105.20±19.87	67.64±10.92
Feed S	ND	0.66±0.24	2.77±0.55	20.64±3.06	277.17±70.66	0.73±0.24	100.38±19.03	64.11±7.32
Feed T	ND	0.51±0.06	0.73±0.40	30.66±10.75	189.65±26.89	0.31±0.00	127.33±27.61	85.82±7.18
Feed V	ND	0.49±0.43	0.49±0.42	26.83±12.55	251.16±22.36	0.61±0.00	82.42±30.20	65.98±17.55
Mean	2.31±1.23	0.43±0.20	1.66±0.82	23.35±5.26	238.09±45.80	0.61±0.26	99.57±15.63	68.21±11.92

ND: Not detected

**Table 3** Concentration ( $\mu\text{g/g}$  dry weight) of heavy metals in Starter feed ratios of the different feed brands

Feed brands	Metals							
	Pb	Cd	Ni	Cu	Fe	Co	Mn	Zn
AS	4.14±0.03	0.64±0.30	1.80±0.26	37.76± 1.33	304.26±16.07	1.25±0.70	101.48±14.78	74.49±9.41
GS	ND	0.02±0.01	3.47±2.15	40.39±3.32	247.65±33.31	ND	182.29±9.81	117.20±0.69
HS	1.28±0.12	0.09±0.03	1.37±0.47	22.83±7.70	215.65±0.23	ND	82.40±5.41	61.24±16.29
LS	0.43±0.16	0.18±0.44	2.13±0.43	16.35±2.30	194.76±6.49	ND	104.92±16.31	65.31±2.11
SS	ND	0.88±0.46	3.17±0.96	24.88±2.50	377.17±12.72	0.48±0.13	121.46±1.71	72.77±0.74
TS	ND	0.52±0.01	0.87±0.51	42.55±13.77	193.35±17.69	ND	163.66±3.33	91.82±8.11
VS	ND	0.16±0.03	0.06±0.01	13.43±0.94	217.78±5.23	ND	41.19±0.06	41.56±0.13
Mean	1.95±1.94	0.36±0.33	1.84±1.21	28.31±11.86	250.09±67.82	0.87±0.54	113.9±47.83	74.91±24.05

Values are mean  $\pm$  standard deviation of triplicate determinations (n = 3); ND: Not detected**Table 4** Concentration ( $\mu\text{g/g}$  dry weight) of heavy metals in Grower feed ratios of the different feed brands

Feed brands	Metals							
	Pb	Cd	Ni	Cu	Fe	Co	Mn	Zn
AG	1.53±1.14	0.41±0.22	2.56±1.70	20.20±0.66	242.53±7.74	ND	90.01±5.82	49.60±1.83
GG	ND	ND	1.43±0.70	24.82±1.21	173.61± 7.35	ND	73.79±14.38	65.54±8.71
HG	ND	ND	2.87±2.55	11.23±1.99	280.76±7.64	0.44±0.14	75.50±0.47	61.23±24.04
LG	7.75±1.12	0.62±0.24	1.90±0.21	13.88 ±1.33	182.12±16.54	ND	77.45±1.03	53.32±2.08
SG	ND	0.31±0.06	2.38±2.27	19.57±3.40	222.76±21.83	0.97±0.27	110.67±2.47	67.52±1.16
TG	ND	0.52±0.11	1.04±0.77	20.37±3.21	174.66±22.77	ND	97.89±7.96	76.53±5.68
VG	ND	0.82±0.09	0.27±0.13	19.03±0.62	265.03±2.63	0.61±0.66	112.58±7.06	64.89±0.37
Mean	4.64±4.40	0.54±0.20	1.78±0.92	18.44±4.51	220.21±44.49	0.67±0.27	91.13±16.44	62.66±9.03

Values are mean  $\pm$  standard deviation of triplicate determinations (n = 3); ND: Not detected

The heavy metal contents in starter feed rations of the different feed brands revealed that iron concentrations were highest in all the brands. The Feed S and Feed T starter rations gave the highest and lowest iron concentrations of  $377.17 \pm 12.72 \mu\text{g/g}$  dry weight and  $193.35 \pm 17.69 \mu\text{g/g}$  dry weight respectively (Table 3). Also, the Feed A starter ration gave the highest value in Pb while the lowest value was recorded in Feed L starter ration. Like in other rations, the iron concentrations in the grower feed rations were the highest. Generally, the Pb and Cd concentrations were quite low where they were observed except in Feed L where the concentration is very high (Table 4).

The heavy metal concentrations in Grower feed rations are in the order:  $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Pb} > \text{Ni} > \text{Co} > \text{Cd}$ . The highest concentrations of Cd ( $0.90 \pm 0.16 \mu\text{g/g}$  dry weight) and Cu ( $39.24 \pm 0.09 \mu\text{g/g}$  dry weight) in finisher feed ration were found in Feed V brand while the lowest concentrations were observed in Feed G and Feed H rations respectively (Table 5). Lead was not detected in Feeds H, S, T and V finisher rations. The results of heavy metal contents in layer feed rations reveals that the concentrations of Fe in all the feed brands were consistently higher than other heavy metals under study with Feed H layer ration having the highest concentration of  $363.65 \pm 7.60 \mu\text{g/g}$  dry weight (Table 6). On the other hand, the lowest concentrations of Ni, Fe, Mn and Cu, Co, Zn were detected in Feed G and Feed H rations respectively (Table 6).

**Table 5** Concentration ( $\mu\text{g/g}$  dry weight) of heavy metals in Finisher feed rations of the different feed brands

	Metals							
Feed brands	Pb	Cd	Ni	Cu	Fe	Co	Mn	Zn
AF	$0.44 \pm 0.20$	$0.45 \pm 0.31$	$1.59 \pm 0.15$	$25.39 \pm 6.97$	$195.44 \pm 2.40$	$0.86 \pm 0.33$	$103.29 \pm 7.49$	$63.84 \pm 0.30$
GF	$2.43 \pm 2.31$	$0.07 \pm 0.02$	$2.07 \pm 0.70$	$16.77 \pm 3.87$	$84.04 \pm 54.52$	ND	$85.76 \pm 0.31$	$79.27 \pm 8.77$
HF	ND	ND	$1.46 \pm 0.37$	$13.16 \pm 5.37$	$272.32 \pm 4.43$	ND	$77.54 \pm 6.71$	$39.64 \pm 2.10$
LF	$3.89 \pm 1.93$	$0.43 \pm 0.04$	$1.76 \pm 1.16$	$19.63 \pm 0.01$	$233.22 \pm 37.07$	ND	$122.48 \pm 1.36$	$73.94 \pm 2.99$
SF	ND	$0.73 \pm 0.04$	ND	$20.47 \pm 0.23$	$232.24 \pm 13.07$	ND	$80.01 \pm 4.04$	$57.29 \pm 2.60$
TF	ND	$0.43 \pm 0.26$	$0.28 \pm 0.20$	$36.87 \pm 12.08$	$164.75 \pm 1.75$	$0.31 \pm 0.14$	$117.66 \pm 0.45$	$91.02 \pm 6.82$
VF	ND	$0.90 \pm 0.16$	$1.02 \pm 0.15$	$39.24 \pm 0.09$	$262.18 \pm 20.39$	ND	$93.63 \pm 3.52$	$78.33 \pm 0.83$
Mean	$2.25 \pm 1.73$	$0.50 \pm 0.29$	$1.36 \pm 0.63$	$24.50 \pm 10.00$	$206.31 \pm 65.41$	$0.58 \pm 0.39$	$97.20 \pm 17.89$	$69.05 \pm 16.97$

Values are mean  $\pm$  standard deviation of triplicate determinations (n = 3); ND: Not detected

**Table 6** Concentration ( $\mu\text{g/g}$  dry weight) of heavy metals in Layer feed rations of the different feed brands

	Metals							
Feed brands	Pb	Cd	Ni	Cu	Fe	Co	Mn	Zn
AL	$0.13 \pm 0.05$	$0.63 \pm 0.16$	$3.45 \pm 0.61$	$18.31 \pm 1.78$	$341.56 \pm 2.04$	$0.83 \pm 0.22$	$92.98 \pm 13.33$	$65.96 \pm 0.10$
GL	$2.35 \pm 1.61$	$0.52 \pm 0.24$	$0.28 \pm 0.18$	$21.08 \pm 1.42$	$153.95 \pm 11.16$	ND	$72.91 \pm 9.84$	$60.88 \pm 8.78$
HL	ND	ND	$1.57 \pm 0.88$	$15.11 \pm 5.16$	$363.65 \pm 7.60$	$0.19 \pm 0.06$	$88.54 \pm 8.27$	$36.88 \pm 7.24$
LL	ND	$0.82 \pm 0.39$	$0.83 \pm 0.29$	$24.40 \pm 2.12$	$309.00 \pm 13.38$	$0.71 \pm 0.21$	$115.95 \pm 14.12$	$78.00 \pm 2.53$
SL	ND	$0.69 \pm 0.10$	ND	$17.64 \pm 1.17$	$276.51 \pm 19.86$	$0.75 \pm 0.36$	$89.39 \pm 10.43$	$58.87 \pm 6.33$
TL	ND	$0.55 \pm 0.42$	ND	$22.87 \pm 0.44$	$225.86 \pm 14.74$	ND	$130.10 \pm 10.86$	$83.90 \pm 4.27$
VL	ND	$0.09 \pm 0.01$	$0.61 \pm 0.26$	$35.64 \pm 3.67$	$259.65 \pm 16.20$	ND	$82.27 \pm 6.73$	$79.14 \pm 2.66$
Mean	$1.24 \pm 1.57$	$0.55 \pm 0.25$	$1.35 \pm 1.27$	$22.15 \pm 6.25$	$275.74 \pm 66.28$	$0.62 \pm 0.29$	$96.02 \pm 18.48$	$66.23 \pm 14.94$

Values are mean  $\pm$  standard deviation of triplicate determinations (n = 3); ND: Not detected

A comparison of the results of heavy metal concentrations in different poultry feed rations is summarized in Table 7. The starter feed ration was observed with the highest mean concentrations of  $1.84 \pm 1.21$ ,  $28.31 \pm 11.86$ ,  $0.87 \pm 0.54$ ,  $113.91 \pm 47.83$  and  $74.91 \pm 24.05 \mu\text{g/g}$  dry weight for Ni, Cu, Co, Mn and Zn metals respectively. The layer feed ration contained Cd ( $0.55 \pm 0.25 \mu\text{g/g}$  dry weight) and Fe ( $275.74 \pm 66.28 \mu\text{g/g}$  dry weight) the most while the highest Pb

concentration of  $4.64 \pm 4.40 \mu\text{g/g}$  dry weight was found in grower feed ration. Overall, metal accumulation in the feed rations varied from metal to metal as shown in the results (Table 7). Cu, Mn and Zn followed similar pattern: starter > finisher > layer > grower. The trend followed by other metals are Pb (grower > finisher > starter > layer), Cd (layer > grower > finisher > starter), Ni (starter > grower > finisher > layer), Fe (layer > starter > grower > finisher) and Co (starter > grower > layer > finisher).

**Table 7** Comparison of mean concentration ( $\mu\text{g/g}$  dry weight) of heavy metals in the different poultry feed rations

Metals	Feed rations			
	Starter	Grower	Finisher	Layer
Pb	$1.95 \pm 1.94^a$	$4.64 \pm 4.40$	$2.25 \pm 1.73$	$1.24 \pm 1.57$
	(ND - 4.14) <sup>b</sup>	(ND - 7.75)	(ND - 3.89)	(ND - 2.35)
Cd	$0.36 \pm 0.33$	$0.54 \pm 0.20$	$0.50 \pm 0.29$	$0.55 \pm 0.25$
	(0.02 - 0.88)	(ND - 0.82)	(ND - 0.90)	(ND - 0.82)
Ni	$1.84 \pm 1.21$	$1.78 \pm 0.92$	$1.36 \pm 0.63$	$1.35 \pm 1.27$
	(0.06 - 3.47)	(0.27 - 2.87)	(ND - 2.07)	(ND - 3.45)
Cu	$28.31 \pm 11.86$	$18.44 \pm 4.51$	$24.50 \pm 10.00$	$22.15 \pm 6.25$
	(13.43 - 42.55)	(11.23 - 24.82)	(13.16 - 39.24)	(15.11 - 35.64)
Fe	$250.09 \pm 67.82$	$220.21 \pm 44.49$	$206.31 \pm 65.41$	$275.74 \pm 66.28$
	(193.35 - 377.17)	(173.61 - 280.76)	(84.04 - 272.32)	(153.95 - 363.65)
Co	$0.87 \pm 0.54$	$0.67 \pm 0.27$	$0.58 \pm 0.39$	$0.62 \pm 0.29$
	(ND - 1.25)	(ND - 0.97)	(ND - 0.86)	(ND - 0.83)
Mn	$113.91 \pm 47.83$	$91.13 \pm 16.44$	$97.20 \pm 17.89$	$96.02 \pm 18.48$
	(41.19 - 182.29)	(73.79 - 112.58)	(77.54 - 117.66)	(72.91 - 130.10)
Zn	$74.91 \pm 24.05$	$62.66 \pm 9.03$	$69.05 \pm 16.97$	$66.23 \pm 14.94$
	(41.56 - 117.20)	(49.60 - 76.53)	(39.64 - 91.02)	(36.88 - 83.90)

a. Mean concentrations of metals in feed rations; b. Range of metal concentrations in feed rations

#### 4. Discussion

Generally, the order of heavy metal concentrations in the poultry feeds are Fe > Mn > Zn > Cu > Ni > Pb > Co > Cd. The low levels of lead and cadmium in the feed is expected because these elements have toxic properties on plants and animals and their presence could result in deleterious effect on man who is at the top of the food chain when he consumes food containing high level of these metals. The contaminations of Pb and Cd in the feeds could be due to the use of contaminated feed ingredients in the production of the feeds. Also, the presence of cadmium in the feeds cannot be excluded from the use of phosphate fertilizers in agricultural production of maize [34].

Lead concentration (dry weight) in the different feed brands ranged between ND -  $4.02 \pm 3.66 \mu\text{g/g}$  with a mean value of  $2.31 \pm 1.23 \mu\text{g/g}$  (Table 2). This value is lower than the ranges of 23.2 - 32.6 mg/kg, 1.10 - 7.85 mg/kg and 2.33 - 7.90 ppm obtained by Mahesar et al. [35], Okoye et al. [28] and Imran et al. [36] respectively. On the contrary, Suleiman et al. [37] reported lower lead contents in poultry feeds. The concentration of Pb in the feed sample is below the 5 mg/kg maximum permissible limit in feeds by FAO/WHO [38]. Comparison of metal concentration in the poultry feed rations, revealed that grower and layer feed rations contained the highest and lowest Pb concentrations (Table 7). Lead is a non-essential element that has direct health concern on humans [39,40]. Anthropogenic activities which include combustion of fossil fuels, agricultural activities and industrial emission are the major sources of lead.

The Cd concentrations measured in the poultry feed brands ranged from  $0.09 \pm 0.01$  -  $0.66 \pm 0.24 \mu\text{g/g}$  dry weight having a mean value of  $0.43 \pm 0.20 \mu\text{g/g}$  dry weight (Table 2). This value is lower than the maximum permissible limit of 1 mg/kg in feeds given by FAO/WHO [38]. Previous studies by Mahesar et al. [35], Zhang et al. [27], Mohammad et al. [26]

and Imran et al. [36] reported higher Cd concentrations in the ranges of 3.8 - 33.8 mg/kg, ND - 8.00 mg/kg, 3.33 - 16.67 mg/kg and 0.11 - 1.41 mg/kg respectively. In Suleiman et al. [37] a low cadmium content of 0.01 - 0.55 µg/ml which is similar to the values obtained in this study was reported in feeds. Cadmium mimics other divalent metals that are necessary for a variety of biological processes but has no recognized biological function in humans or animals [41,42].

The levels of nickel detected in the different feed brands fell between the ranges of  $0.49 \pm 0.42$  and  $2.77 \pm 0.55$  µg/g dry weight with a mean value of  $1.66 \pm 0.82$  µg/g dry weight (Table 2). The concentrations of Ni obtained in this study were generally higher than 0.5 mg/kg maximum permissible limit in feed by FAO/WHO [43] and WHO [44]. Several studies have reported nickel in feed samples; Imran et al. [36], Islam et al. [45], Mohammad et al. [26] and Okoye et al. [28] observed high nickel concentration ranges of 2.91 - 5.52 ppm, 0.0125 - 5.1625 ppm, 11.33 - 64.33 mg/kg and 2.250 - 4.875 mg/kg respectively in their studies, while Suleiman et al. [37] observed low nickel level of 0.004 - 0.250 µg/ml. Although nickel has been found to serve biological functions in some animals and plants, its essentiality in humans has not been established [46]. Nickel is one of the naturally occurring metals, in addition, it enters the environment through the incineration of waste, burning of fossil fuels and coal, nickel mining and electroplating [47].

The concentration of copper in the different feed brands ranged between  $15.58 \pm 5.08$  and  $30.66 \pm 10.75$  µg/g dry weight with a mean value of  $23.35 \pm 5.26$  µg/g dry weight (Table 2). These values are higher than 8 mg/kg recommended in poultry diets by NRC [48]. The levels of copper observed in the poultry feeds were within the ranges though lower than 12.3 - 65.8 mg/kg, 2.88 - 98.08 mg/kg and 45.67 - 98.67 mg/kg published in Mahesar et al. [35], Zhang et al. [27] and Mohammad et al. [26] whereas Imran et al. [36] and Suleiman et al. [37] reported lower copper concentrations in feeds. The highest and lowest copper concentrations in the feed rations were found in starter and grower feed rations respectively (Table 7). These values exceeded the required copper contents of 4 mg/kg for starter and finisher rations and 6 mg/kg for grower and layer rations set by SON [8]. Copper is considered essential to life but toxic to biological systems in higher concentrations. Food can be contaminated with copper through processing, agricultural inputs, metal-based work, and uptake by plants from contaminated soil [49].

Iron concentration in the different feed brands was observed to range between  $164.81 \pm 67.28$  and  $283.09 \pm 60.99$  µg/g dry weight with a mean value of  $238.09 \pm 45.80$  µg/g dry weight (Table 2). The iron contents in the feeds brands were all higher than the 80 mg/kg recommended limit in feeds by NRC [48]. The high concentrations of Fe in the poultry feeds suggests excess mineral supplementation in the feeds. In previous studies, iron content in poultry feeds were reported in the ranges of 50.575 - 170.075 mg/kg and 76 - 116 ppm by Okoye et al. [28] and Imran et al. [36] respectively. On the contrary, Anas et al. [50], Kabir et al. [51] and Mohammad et al. [26] obtained higher concentrations of 113 - 718 ppm, 231.5 - 435.8 ppm and 28.23 - 1026.67 mg/kg of iron in poultry feeds respectively. The mean levels of iron in the various feed rations were higher than the recommended nutrient requirements of 60 mg/kg by SON [8] for starter and finisher feed rations and 30 mg/kg for grower and layer feeds (Table 7). This revealed that Fe concentrations in the feeds were 3 - 5 times higher than the values required by the chicken for healthy development. Iron is an essential element and its presence in adequate amount in diet is very important.

The concentrations of cobalt in the different feed brands ranged from ND to 0.98 µg/g dry weight with a mean concentration of  $0.61 \pm 0.26$  µg/g dry weight (Table 2). This value is within the recommended limit of 1 mg/kg in feeds set by EFSA [41]. A study by Okoye et al. [28] recorded cobalt levels of 0.613 - 3.200 mg/kg in feeds. Ukpe and Chokor [52] observed higher mean concentration of  $0.390 \pm 0.009$  mg/g than the present study.

Manganese is an element that is required in the diet and it plays a key role in the enzyme systems involved in carbohydrate and lipid metabolism [53]. The mean concentration of manganese in the feed brands was found to be  $99.57 \pm 15.63$  µg/g dry weight (Table 2) which is higher than 20 mg/kg and 60 mg/kg NRC [48] recommended values of Mn for laying hens and broilers respectively. This may be due to mineral supplementation. Mohammad et al. [26] obtained high levels of manganese in poultry feeds than the present study while Okoye et al. [28], Imran et al. [36], Suleiman et al. [37] and Anas et al. [50] observed lower manganese concentrations in feeds. Furthermore, the starter and grower feed rations recorded the highest and lowest mean concentrations of manganese as shown in Table 7. All the values were above the SON [8] manganese requirement of 30 mg/kg in starter and finisher rations and 60 mg/kg in grower and layer rations.

The concentration (dry weight) of zinc in the poultry feed brands was observed to be in the range of 49.75 - 85.82 µg/g with a mean concentration of  $68.21 \pm 11.92$  µg/g (Table 2). The zinc concentration observed in this study were higher than the 40 mg/kg NRC [48] recommended limit in poultry feeds. This reflects excess addition of Zn mineral to meet animal nutrient requirements. Zinc is a trace element necessary for cell growth and division, protein synthesis, wound healing, immune function, enzyme reactions and DNA synthesis [54,55]. Zinc deficiency in young chicks can cause loss of appetite, retarded growth, scaling of the skin (especially on the feet), shortening and thickening of leg bones and



enlargement of the hock joint, very poor feathering, and in extreme cases, mortality [56]. Some authors, Anas et al. [50] and Zhang et al. [27] recorded zinc concentrations higher than the present study. However, Mohammad et al. [26], Okoye et al. [28], Imran et al. [36] and Suleiman et al. [37] observed lower concentrations of Zn in poultry feeds. Zinc was detected in some feed rations in levels higher than the mineral requirement of 35 mg/kg in starter and finisher rations and 60 mg/kg in grower and layer feed rations as recommended by SON [8] (Table 7). The Zn concentrations in Feeds A and L grower feed rations and Feeds H and S layer rations were within the SON [8] acceptable levels (Tables 4,6). Zn concentrations in the feed rations studied were lower than the values estimated by Nicholson et al. [57] in broiler starter (148 mg/kg), grower (118 mg/kg), finisher (135 mg/kg) and layer (153 mg/kg).

The ANOVA analysis result for the comparison of heavy metal concentrations in the poultry feed brands returned a  $p < 0.05$  implying there was a statistical significant difference in the metal content distributions in the feed brands. Hence, a Post hoc analysis was conducted using Duncan multiple range test to determine the particular metal(s) with variant distribution. It was observed that Pb, Cd, Ni and Co are of similar distribution in the feed samples. Their presence in feeds could be mainly as result of anthropogenic activities along the line of feed formulation (harvesting, storage, processing, transportation etc.). Pb and Cd are toxic metals and are not beneficial in body metabolism. However, Cu, Mn, Fe and Zn each have unique metal distribution. These are trace metals and due to supplementation in feeds exhibited unique pattern in distribution as observed in the feed brands.

Pearson correlation analysis on the heavy metals in poultry feeds showed a significant ( $p < 0.05$ ) positive weak correlation ( $r = 0.301$ ) between iron and cadmium. Also, there was positive strong correlations between Co/Ni (0.658), Mn/Cu ( $r = 0.566$ ), Zn/Cu ( $r = 0.683$ ) and Zn/Mn ( $r = 0.758$ ) in the feeds at significance level  $p < 0.01$ . The positive correlations suggest that these elements could have had the same pathway into the feeds. Copper, manganese and zinc are micro nutrients required by poultry and are usually added to feeds through premixes.

## 5. Conclusion

The concentrations of iron, copper manganese and zinc in the studied poultry feed were higher than the maximum recommended limits set by FAO/WHO, NRC and SON. This could probably be due to deliberate addition of these essential micronutrients to meet animal nutrient requirements. For the poultry feed rations, the levels of Zn, Cu and Mn followed the pattern: starter > finisher > layer > grower, while Fe followed the pattern: layer > starter > grower > finisher. Also, lead and cadmium were detected in elevated levels in some feed samples. Overall, the poultry feeds assessed were contaminated with heavy metals, hence, there is need for regular monitoring of commercial feeds by regulatory authorities to ensure that concentrations of the heavy metals that may be present are below the maximum level. Besides, enforcement of good manufacturing practices should be encouraged.

## Compliance with ethical standards

### *Acknowledgments*

The authors wish to express their thanks to the management of Chemistry Advanced Research Center, Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria for facilitating the research by granting their laboratory usage. We also thank Mr Bwai David Macham for his assistance during instrumental analysis.

### *Disclosure of conflict of interest*

No conflict of interest was declared by the authors.

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