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# Assessment of aflatoxin and heavy metals levels in maize and poultry feeds from Delta State, Nigeria

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## Abstract

The aim of this study was to determine the concentration of total aflatoxin and heavy metals (lead cadmium, chromium and copper) in poultry feed and ingredients from two regions (north and central) in Delta State, Nigeria. A total of 120 samples collected (comprising of maize, soybean meal, layers mash and broiler finisher) directly from poultry farms, feed mills and poultry feed dealers were analysed for moisture content, total aflatoxin and heavy metals (lead, cadmium, chromium and copper) concentrations. Moisture content was analysed using standard Association of Official Agricultural Chemists method, while total aflatoxin and heavy metals concentrations were determined by enzyme-linked immunosorbent assay and atomic absorption spectrometry, respectively. Total aflatoxins concentrations ranged from 12.0 to 20 µg/kg and 21 to 31 µg/kg in samples from north and central region, respectively. Apart from maize, aflatoxin content in other samples from the north was marginally lower (18–20 µg/kg) than permitted levels. On the other hand, aflatoxin levels in samples from the central were all above tolerable limits. Copper content in samples was less than permissible limit, while 44.4, 29.1 and 21% of samples had concentrations of lead, cadmium and chromium, respectively, above permissible limit. The presence of aflatoxin and lead in poultry feeds above permissible limits of 20 µg/kg and 5 mg/kg, respectively, may pose a risk for animal productivity and human health.

**Keywords** Aflatoxins · Finished poultry feeds · Heavy metals · Permissible limits · Poultry feed ingredients

## Introduction

In recent years, the Nigerian poultry industry has been rapidly expanding and has become one of the most commercialized sub-sectors of Nigerian agriculture (United States Department of Agriculture USDA 2010). However, poultry feeds which comprises of maize, peanut meal, soya bean meal and mixtures of maize, groundnut cake and other crops

have been associated with mycotoxin contamination during crop production and storage (Getachew et al. 2018), with limited attention given to this by local poultry farmers and regulatory bodies. Food and Agriculture Organization report states that about up to a quarter of the world's growing crops are affected by mycotoxins each year (Imade et al. 2021). In a recent study on mycotoxin contamination in maize and finished feed samples from 8 African countries, 33.3% of maize samples and 54.4% of finished feed samples were above the European regulatory limit of 20 ng/g aflatoxins (Gruber-Dorninger et al. 2018). The maximum permitted level for aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is 20 µg/kg and recommended values for deoxynivalenol (DON), zearalenone (ZEN), T-2, ochratoxin A (OTA) and fumonisins (FB1 + FB2) are 5, 0.25, 0.25, 0.1 and 20 mg/kg, respectively, in poultry feed (Commission 2006). For poultry feed ingredients, (maize soybean meal, etc.) permitted levels of mycotoxins range from 0.02 to 0.1 mg/kg (Magnoli et al. 2019).

The losses resulting from mycotoxin contamination are numerous, ranging from loss of value of commodities,

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which translates to trade and economic losses, decline in animal productivity and ultimately human health cost which in fatal cases might result in death (Bhat Ramesh and Vasanthi 2003; Rodrigues et al. 2011; Shephard 2008). Aflatoxins (AF) formed by strains of *Aspergillus flavus*, *A. flavus* subsp. *parasiticus* and *Aspergillus nomius* have received greater attention than any other mycotoxins, and among the types of AFs reported, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), aflatoxin G<sub>1</sub> (AFG<sub>1</sub>), aflatoxin B<sub>2</sub> (AFB<sub>2</sub>) and aflatoxin G<sub>2</sub> (AFG<sub>2</sub>) are the most toxic to human health (Nasir et al. 2021). The effect of domestic animals ingesting feed infected with even sub-lethal doses of AF includes reduced output and reproduction, increased vulnerability to diseases and decreased quality of foods produced (Joint et al. 2017).

According to International Agency of Research on Cancer Agency for Research on Cancer (IARC), AF, especially AFB<sub>1</sub>, is classified as group 1 human carcinogen (Nasir et al. 2021; WHO 1993). AF residues are predominant in eggs, milk, meat flesh and organs, and their consumption by humans is a major route of mycotoxin intake causing a wide range of harmful effects (Adegbeye et al. 2020; Li et al. 2021). There are a number of environmental conditions that favour the growth and production of mycotoxins, namely humidity, temperature, moisture and handling conditions during harvest and storage (Mutegi et al. 2013). In Nigeria, humid environmental circumstances (average RH: 84% and 33 °C) coupled with improper and inefficient storage practices present ideal conditions for fungal growth and then, mycotoxin production. The risk of mycotoxin contamination in poultry feeds in Nigeria is further heightened by lack of strict regulations regarding feeds and feed materials in the poultry sector (Rodrigues et al. 2011). Therefore, extensive studies on the prevalence of AF contamination in poultry feeds from different geographical regions in Nigeria are needed for the purpose of legislation and adopting effective strategies aimed at controlling its occurrence (Ezekiel et al. 2012).

Heavy metals are a group of contaminants in feed materials and animal feeds. These heavy metals, including cadmium (Cd), lead (Pb), copper (Cu) and chromium (Cr), have specificity density in excess of 5 g/cms and are toxic or poisonous even at low concentrations (Duruibe et al. 2007; Järup 2003). The route through which these toxic substances contaminate plant materials includes the use of polluted water for irrigation, fertilisers, toxic pesticides and industrial activities such as mineral and oil exploration and post-harvest contamination (Alkhalaf et al. 2010). Farm animals ingest these contaminated plant materials and subsequently pass these toxic substances up the food chain to humans via animal products (Hinton 2000). The adverse effects associated with heavy metal intake include damage to the lungs, kidneys, tissues and skeletal system, osteoporosis and, in

some instances, cause cancer of the lungs and blood (Akhtar et al. 2017; Blair and Lamb 2017; Ismail et al. 2014; Rebelo and Caldas 2016).

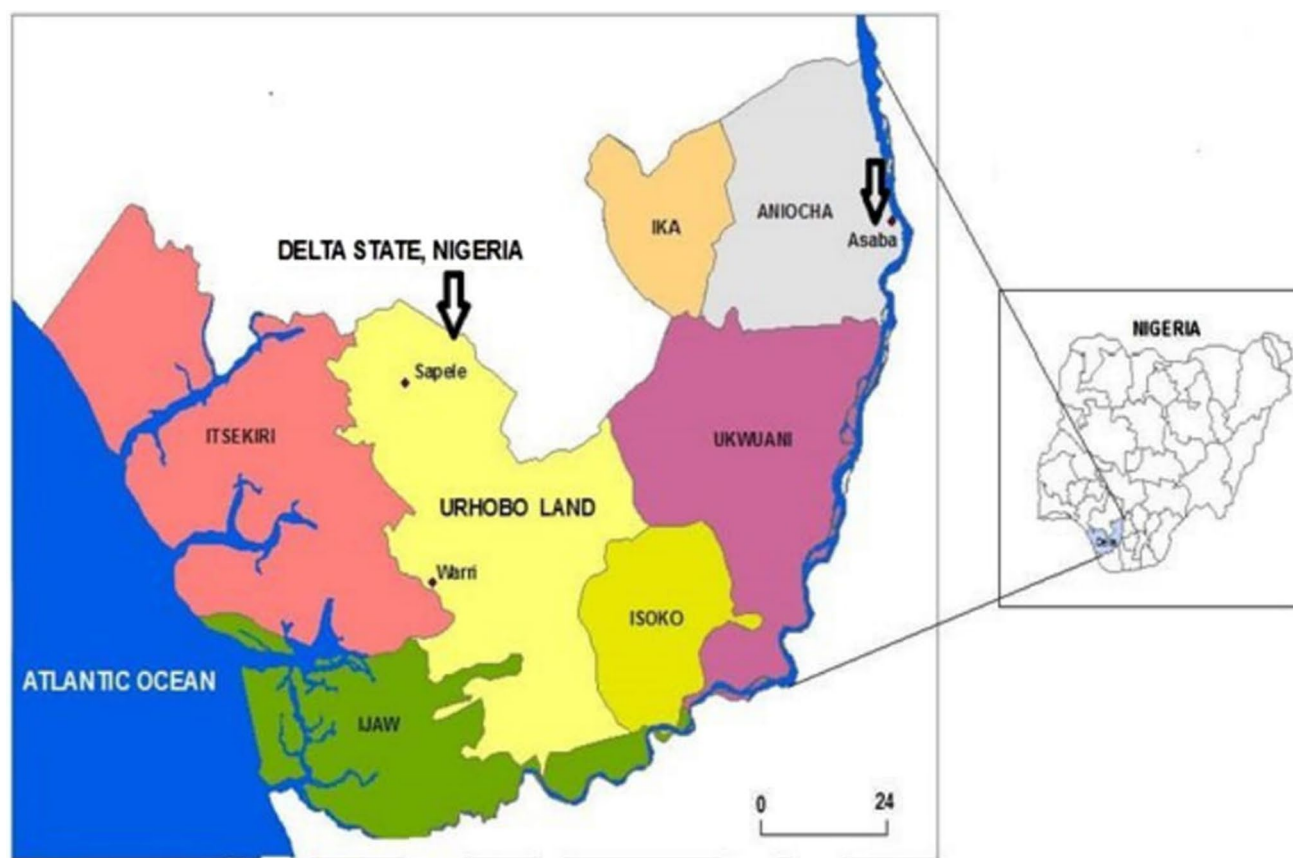
Although there are several studies on the occurrence of heavy metals in animal products (Cang et al. 2004; Javed et al. 2009; Tajkarimi et al. 2008; Vidovic et al. 2005), data on the prevalence of heavy metals contamination in poultry feeds and ingredients in Nigeria are scanty. The ubiquitous nature of food-borne mycotoxins (e.g. aflatoxins) and environmental contaminants (e.g. heavy metals) makes possible their entry into the animal portion and food chain either during the harvest, storage or processing period. In addition, sewage sludge and sewage water are commonly applied in most farms and pastures by subsistence farmers which multiplies the toxic metals in the soil environment and forage pastures in these farms. In addition, the oil exploration and mineral mining in the Niger Delta region of Nigeria result in the contamination of farm settlements by heavy metals. These are either taken up by plants or contaminate the farm produce and thereafter transferred into the food chain. Since such co-contamination is inevitable and could increase the possibility of inducing toxic effects in humans and animals, it is important to evaluate such co-contamination to understand the risk posed by such mixtures of contaminants spanning different chemical groups. Therefore, the aim of this study was to assess the co-contamination of total AF and heavy (Cd, Pb, Cr and Cu) of poultry feeds and ingredients from two regions (North & Central) between the months of October 2019–January 2020 in Delta State, Nigeria.

## Materials and methods

### Sampling method

A total of 120 poultry feeds and poultry feed ingredients from two regions (North and Central) of Delta State, Nigeria, comprising of maize ( $n=30$ ), broiler finisher ( $n=30$ ), layers mash ( $n=30$ ) and soybean meal ( $n=30$ ) were randomly collected from different poultry farmers ( $n=5$ ), feed mills ( $n=5$ ) and feed dealers ( $n=5$ ) for each sample group in each region bringing the total number of samples collected from each region to be 60 each. In addition, crude oil exploration activities are more prevalent in the central than northern region (Fig. 1). The feed samples were properly packaged in separate clean zip-lock polythene bags, labelled and transported to the laboratory and stored frozen ( $-20\text{ }^{\circ}\text{C}$ ) to prevent further production of the metabolite by fungi and bacteria prior to AF and heavy metal analysis.





**Fig. 1** Locations of sampled sites in Central and Northern regions of Delta State, Nigeria Asaba is located on Latitude:  $6^{\circ}11'53''$  N, and Longitude:  $6^{\circ}43'54''$  E. The elevation above sea level is 46 m (150 ft.). The least amount of rainfall occurs in December (11 mm), while September has the highest rainfall (313 mm). The average RH of

Asaba is around 78%. Sapele is located on Latitude  $5^{\circ}53'38''$  N and Longitude  $5^{\circ}40'35''$  E. The elevation above sea level is 11 m (36 ft.). Rainfall is at its peak between June and July with an average rainfall of 431 mm, while the lowest rainfall of 7.62 mm occurs in January. The average RH of Sapele is around 85%

### Determination of moisture content

The moisture content of samples was determined using the standard oven method (Association of Official Agricultural Chemists AOAC 1990). The frozen samples were allowed to thaw at room temperature. Thereafter, they were weighed, dried in triplicate at  $105^{\circ}\text{C}$  to a constant weight, and the average moisture content was determined on a percentage dry basis.

### AF extraction and analysis

The extraction and analysis of AF in samples were evaluated after slight modifications according to the method of Kana et al. (2013). Twenty-five (25 g) of all samples collected were measured in triplicates and ground using a roller mill and poured into a conical flask. For extraction of AF, 25 mL of 70% methanol solution was poured into each of the flask containing the milled sample and thoroughly mixed using a rotatory shaker operated at 225 rpm

for 4 min at  $25^{\circ}\text{C}$ . The mixture was passed through a filter paper (Whatman No. 1) and 2 mL of the filtrate pipetted into a 10 mL flask and filled to the mark with distilled water. For sample clean up, the diluted extract was passed through an Aflatest®-P affinity column (VICAM) at a rate of 1–2 drops/second and to remove impurities, and the column was rinsed with distilled water. HPLC grade methanol was then passed through the affinity column at a rate of 1–2 drops/second for elution of bound AF and the eluate collected in a cuvette. For AF analysis, 1 mL of Aflatest® developer solution (VICAM) was added to the eluate and mixed thoroughly. The cuvette was then placed in a micro-titer plate reader (BioTek) earlier calibrated according to the manufacturer's protocol, and after 60 s, total AF concentration ( $B1 + B2 + G1 + G2$ ) ( $\mu\text{g}/\text{kg}$ ) in the samples was read and recorded. The analysis of AF from each sample was carried out in triplicates.

The linearity of the method was tested by running AF standard in the range of 0.0–40  $\mu\text{g}/\text{kg}$  (0, 2, 10, 20 and 40  $\mu\text{g}/\text{kg}$ ), and a correlation coefficient ( $R^2$ ) of 0.9913 was



obtained. The method was reproducible, and the range of CV for inter-day precision was 0.35–4.01. The limit of detection (LOD) and limit of quantification (LOQ) of the method were 3 and 4 µg/kg, respectively. The recovery of method of aflatoxin analysis was evaluated by spiking uncontaminated maize samples with standard aflatoxin concentrations of 20 and 30 µg/kg and the recovery of analyte was 91.6 & 107.2% respectively.

### Determination of heavy metals in feed samples

Feed samples were analysed for lead, cadmium and copper and chromium contents using atomic absorption spectrophotometry (AAS) as described by Ifie et al. (2021). For the destruction of organic matter, the dry ashing method was employed. The samples were placed in partially covered and dried in a convention oven set at 100–105 °C overnight (16 h). To aid the ashing process, magnesium nitrate was added to the dried sample at approximately 1:1 ratio. Samples were burnt on a hot plate (2 h), and ashing was performed in a muffle furnace at 470 °C for 16 h. Ash was extracted with 20% (v/v) redistilled nitric acid and filled to a definite volume with 1% nitric acid (v/v) for analysis. An atomic absorption spectrophotometer (model 2380, PerkinElmer, Waltham, MA) with flame atomization (air-acetylene) 60–20, equipped with a burner and a deuterium lamp for background correction, was employed for determination of concentrations of toxic metals. The extraction of ash from each sample was carried out in triplicates.

The method for analysis of heavy in samples was evaluated for linearity precision and accuracy. For each heavy, five calibrations of pure commercial standards of lead, cadmium copper and chromium ranging 0.1–8.0 mg/kg were prepared to evaluate the linearity of the method. The value of the coefficient of correlation ( $r$ ) calculated for lead, cadmium, copper and chromium was 0.9963, 0.9999, 1.000 and 0.9983, respectively. Regarding accuracy, the test of accuracy was done by the standard addition method. Three concentration of standard solution (0.5, 1.0 and 2.0 mg/kg) were added to the sample and the % recovery calculated ranged from 96.8 to 99.3%. The LOD and LLOQ determined were 1 µg/kg and 3 µg/kg, respectively.

### Data analysis

Data analyses were carried out using IBM SPSS software (Version 20.0), and Duncan's multiple range was used to separate differences in means among samples. Statistical analysis was performed comparing the results of all samples together, and values for statistically significant difference were set at  $p < 0.05$ .

## Result and discussion

### Moisture content between samples

One of the major factors influencing AF accumulation in poultry feeds and maize after harvesting is the moisture content. It has been demonstrated that temperatures of 25–30 °C, moisture contents of above 16% and water activity ( $a_w$ ) of above 0.70 encourage fungal growth and mycotoxins production (Mannaa and Kim 2017; Thanushree et al. 2019). The mean scores of moisture content (Table 1) fell within the range of 10.38–12.41% and 11.38–14.75% for samples collected in the northern and central regions, respectively. The results also showed that soybean meal samples (14.75%) from the central region was highest in moisture content. Mogan and Lacy (1988) reported that moisture content  $\leq 15\%$  in maize is safe for storage, although there have been incidences of mould growth within the temperature range of 10–40 °C and equilibrium relative humidity above 70% (D'Mello 1997; Lanyasunya et al. 2005). Kana et al. (2013) analyzed the moisture content of maize, peanut meal and poultry feed mixtures from three agro-ecological zones in Cameroon and reported a range of 10–14% moisture content. In a similar study, the moisture content of poultry feed across five agro-ecological zones in Nigeria ranged from 9.8 to 12.4% (Ezekiel et al. 2014). Comparing the data on moisture content from both regions showed that feed samples from the Delta central presented higher values and this may be due to higher relative humidity found in the central region. The Pearson correlation coefficient of linear regression between the relative humidity (RH) and AF levels

**Table 1** Average moisture content of maize, broiler mash, layers mash and soybean meal collected from two regions in Delta State, Nigeria

Commodity	Zone samples	Number of samples	Moisture content (%) Mean $\pm$ STD	Range (%)
Maize	North	15	12.41 $\pm$ 1.88 <sup>b</sup>	9.8–14.6
	Central	15	11.38 $\pm$ 1.11 <sup>bc</sup>	9.33–13.81
Broiler finisher	North	15	12.23 $\pm$ 1.05 <sup>a</sup>	9.8–15.4
	Central	15	13.10 $\pm$ 1.91 <sup>ab</sup>	9.87–17.89
Layers mash	North	15	11.73 $\pm$ 4.08 <sup>bc</sup>	9.2–15.20
	Central	15	12.79 $\pm$ 3.11 <sup>b</sup>	9.13–19.80
Soy bean meal	North	15	10.38 $\pm$ 2.01 <sup>c</sup>	8.6–12.0
	Central	15	14.75 $\pm$ 2.61 <sup>a</sup>	9.6–18.55

The results represent the average determination of moisture content ( $n=3$ ) of feed samples collected from two regions in Delta State, Nigeria. Statistical analysis was performed comparing the results of all samples together, and values with different letter superscripts on the same column are statistically significantly different ( $p < 0.05$ )





showed a moderate correlation ( $R^2=0.40$ ) with higher AF occurrence of samples in the central region with higher RH.

### AF occurrence of samples

According to the European Union (EU) and Food and Agriculture Organization (FAO) legislation, the maximum tolerable levels for total AF in poultry feeds are 20 µg/kg (FAO 2004; Nasaruddin et al. 2021). In Nigeria, the European Commission and Codex Alimentarius Standards mycotoxin regulations were adopted and are used primarily for export commodities. The maximum acceptable limit for aflatoxins is 10 µg/kg–20 µg/kg, especially for all cereal and all products derived from cereals (Imade et al. 2021).

The result of total AF contamination between samples from both regions is presented in Table 2. AF was detected in ~80% of the samples, and this agrees with other published data on the occurrence of AF contamination in poultry feeds and feed ingredients in Nigeria (Akinmusire et al. 2019; Daodu and Adebawale 2016). Samples collected in the northern region showed no statistical significant difference ( $p > 0.05$ ) in AF contamination between layers mash, broiler finisher and soybean meal. However, maize which recorded the lowest AF contamination (12 µg/kg) was significantly different ( $P=0.027$ ) from soybean. On the contrary, maize from the central region recorded the highest level of AF contamination (31 µg/kg) in samples from both regions. The level of AF contamination of maize in this study coincides with similar research findings on maize AF contamination from three sites in Uganda and South Brazil which ranged from 12.8 to 30 µg/kg and 16.7 to 49.9 µg/kg, respectively (Kaaya and Kyamuhangire 2006; Oliveira et al. 2017). However, in another study, the range of AF contamination in maize from different locations in Nigeria (5–1200 µg/kg), Ghana (4–140 µg/kg) and Kenya (0.5–89 µg/kg) was much higher (Kemboi

et al. 2020; Perrone et al. 2014). Similarly, AFB<sub>1</sub> mean concentration in maize samples from five districts in two agro-ecological zones in Senegal ranged from 5 to 188 µg/kg (Diedhiou et al. 2011). Although there was no significant difference ( $p < 0.05$ ) in AF contamination between layers mash and broiler finisher from the northern region, the levels were higher in layers mash in the central region. This occurrence is contrary to a previous study carried out in Cameroon (Kana et al. 2013), where the mean AF contamination in broiler feed (6.66 µg/kg) was higher than layers mash (11.1 µg/kg).

In a published study (Nemati et al. 2010) from north-western region of Iran, the mean concentrations of AF contamination in broiler feed (11.6 µg/kg) and soybean meal (6.01 µg/kg) were lower than the results (18 and 21 µg/kg broiler finisher and 20 and 30 µg/kg soybean meal) from this study. AF contaminations in this study were also higher than results from Guyana, where mean concentrations of AF in poultry feeds and ingredients ranged from 3.81 to 27.38 µg/kg (Morrison et al. 2017). Similarly, AFB<sub>1</sub> contamination in corn, wheat and commercial poultry feeds purchased from popular markets in Morocco was also lower than our reported values with concentrations ranging from 0.03 to 11.2 µg/kg (Zinedine et al. 2007). Nevertheless, in India, higher incidences of AFs were recorded and were in the range of 10–3500 µg/kg for groundnut cake and sorghum samples; 10–1500 µg/kg in mixed feed samples; 10–300 µg/kg in maize and 10–100 µg/kg in rice bran (Thirumala-Devi et al. 2002). Higher levels (24.–185.25 µg/kg) of AF were also observed in different types of chicken from large-scale and small-scale processors in Uganda (Nakavuma et al. 2020). Aboagye-Nuamah et al. (2021) also reported higher AF values (11.83–88.37 µg/kg) in poultry feed samples from Ghana compared to results obtained in this study. The variations in AF contamination seen can be attributed to differences in geographical location, weather, and farming and

**Table 2** Level of total aflatoxin contamination in maize, broiler mash, layers mash and soybean meal collected from two regions in Delta State, Nigeria

Commodity	Zone	Number of samples	Aflatoxin concentration (ug/kg) Mean ± STD	No/Percentage of samples above-permitted levels	Range (ug/kg)
Maize	North	15	12.0 ± 8.48 <sup>c</sup>	3 (20.0%)	4.0–21.0
	Central	15	31.0 ± 62.22 <sup>a</sup>	8 (53.3%)	9.0–98.0
Broiler finisher	North	15	18.0 ± 6.54 <sup>bc</sup>	3 (20%)	8.0–29.0
	Central	15	21.0 ± 17.54 <sup>b</sup>	10 (66.6%)	11.0–55.0
Layers mash	North	15	19.0 ± 20.74 <sup>bc</sup>	4 (26.67%)	8.0–68.0
	Central	15	30.0 ± 18.15 <sup>a</sup>	12 (80.0%)	14.0–58.0
Soybean meal	North	15	20.0 ± 13.16 <sup>b</sup>	8 (53.3%)	4.0–45.0
	Central	15	30.0 ± 19.80 <sup>a</sup>	13 (86.67%)	11.0–56.0

The results represent the average determination of aflatoxin concentrations (n = 3) of feed samples collected from two regions in Delta State, Nigeria. Statistical analysis was performed comparing the results of all samples together, and values with different letter superscripts on the same column are statistically significantly different ( $p < 0.05$ )



storage practices. Aflatoxin in farm produces can be kept within permissible by adopting best practices such as use of drought-tolerant varieties; timely harvesting before physiological maturity; sorting to remove damaged ears and those having poor husk covering; drying to moisture content of 13%; storage in suitable conditions to keep the crop clean and under condition with minimally proper aeration, or ideally under hermetic conditions (Xu et al. 2021).

### Level of heavy metals occurrence in samples

The mean concentrations of heavy metals contaminations in samples from both regions are presented in Table 3. Currently, Nigeria does not have established maximum admissible levels of chromium, copper, lead and cadmium on feed and foods. However, the EU Directive on toxic substances in animal feed prescribed maximum levels of lead, cadmium and copper as 5, 0.5 and 40 mg kg<sup>-1</sup>, respectively (EU 2013; NRC 2005). Pb is reported to be a commonly encountered

contaminant in the environment (Karak and Bhagat 2010). In this study, the concentration of Pb in samples from central region testing positive (67%) ranged from 2.10 to 13.10 mg/kg, while levels of Pb positive samples (50%) from the northern region ranged from 2.10 to 11.0 mg/kg. Furthermore, positive samples having concentrations above established limits were 75% and 67% for central and northern regions, respectively. In comparison with other published data, Elliot et al. (2017) in an extensive study involving 29 countries from 2009 to 2016 (Asia-Pacific, North and Latin America) analyzed Pb contamination in poultry feeds and reported concentrations as high as 722 mg/kg, far exceeding amounts observed in this research. On the contrary, Eskandari and Pakfetrat (2014) showed that Pb content in all animal feed samples analyzed from Southwestern Iran was lower than 5 mg /kg. Wolf and Cappai (2020) also reported lower Pb concentrations (0.137–0.369 mg/kg) in poultry feed from northern Germany. Although Pb is a contaminant that generally occurs naturally in traces on the Earth's surface, its

**Table 3** Heavy metal contamination in maize, broiler mash, layers mash and soybean meal collected from two regions in Delta State, Nigeria

Samples	Concentration of heavy metals (mg/kg)							
	Lead		Cadmium		Chromium		Copper	
	North	Central	North	Central	North	Central	North	Central
<i>Maize</i>								
Mean	2.87 <sup>c</sup>	1.7 <sup>c</sup>	nd	0.2 <sup>bc</sup>	0.24 <sup>c</sup>	1.40 <sup>a</sup>	1.33 <sup>d</sup>	4.57 <sup>cd</sup>
SD	4.3	1.3	–	0.27	0.35	0.26	1.5	1.21
Min	–	–	–	–	0.001	1.0	–	3.0
Max	8.9	2.9	–	0.7	0.73	1.8	3.5	6.4
% of positive samples above permissible level	100%	–	–	66.6%	33.3%	100%	–	–
<i>Soybean</i>								
Mean	0.7 <sup>c</sup>	0.001 <sup>c</sup>	0.14 <sup>c</sup>	0.08 <sup>c</sup>	0.25 <sup>c</sup>	0.33 <sup>c</sup>	7.40 <sup>bc</sup>	5.01 <sup>cd</sup>
SD	1.15	0.0	0.21	0.07	0.37	0.50	1.07	1.44
Min	–	–	–	–	0.001	0.001	5.9	3.0
Max	2.4	0.001	0.5	0.2	0.8	0.62	8.8	6.2
% of positive samples above permissible level	–	–	33.3%	–	33.3%	33.3%	–	–
<i>Broiler finisher</i>								
Mean	6.6 <sup>ab</sup>	6.8 <sup>ab</sup>	0.24 <sup>bc</sup>	0.59 <sup>a</sup>	0.001 <sup>c</sup>	0.87 <sup>b</sup>	10.58 <sup>b</sup>	7.93 <sup>bc</sup>
SD	5.02	1.26	0.27	0.15	–	0.62	4.93	1.61
Min	–	5.1	0.0	0.4	0.001	0.18	5.4	6.0
Max	11.0	8.4	0.7	0.8	0.001	1.6	17.0	10.0
% of positive samples above permissible level	100%	100%	50%	66.6%	–	66.6%	–	–
<i>Layers mash</i>								
Mean	3.3b <sup>c</sup>	8.2 <sup>a</sup>	0.11 <sup>c</sup>	0.51 <sup>ab</sup>	0.001 <sup>c</sup>	0.20 <sup>c</sup>	4.62 <sup>cd</sup>	15.70 <sup>a</sup>
SD	2.49	3.62	0.17	0.45	–	0.3	1.30	7.67
Min	–	5.20	–	0.10	0.001	0.001	3.4	6.50
Max	5.30	13.10	0.40	1.20	0.001	0.62	6.6	24.70
% of positive samples above permissible level	100%	50%	–	–	–	33.3%	–	–

The results represent the average determination of heavy metal concentrations ( $n=3$ ) of feed samples collected from two regions in Delta State, Nigeria. Statistical analysis was performed comparing all the results of individual heavy metals together, and values with different letter superscripts on the same column are statistically significantly different ( $p<0.05$ )



contamination in plant material increases from industrial and urban pollution (Alloway 2013). In humans, the tolerable limit for Pb is 250 µg/day for adults and 90 µg/day for children (Sharma et al. 2005). Pb toxicity is more common and life threatening in children and aged adults, with neurological and cognitive defects, cardiovascular, renal, gastrointestinal, hepatic and immunological disorders (Dai et al. 2016). In 2010, there was a report of outbreaks of Pb poisoning due to artisanal gold mining in villages in Zamfara State, Nigeria, which led to the death of 118 children less 5 years old (Dooyema et al. 2012). Similarly, co-exposure to Pb and Hg among artisanal gold miners has been reported in Bukkuyum Local Government Area of Zamfara State, Nigeria (Rabiu et al. 2019). In a more recent study, Pona et al. (2021) reported that Pb exposure in Nigeria caused over 2,500 deaths and about 600 thousand disability-adjusted life years (DALYs) lost yearly between 2007 and 2017, with a steady increase in cardiovascular disease and ischaemic heart diseases. Pb toxicity in poultry has also been observed in North-Western Nigeria with concentrations in the tissues ranging between 7.5 mg/kg and 120.5 mg/kg wet weight (Oladipo et al. 2020).

Cadmium is a heavy metal that has no function in the human body and poses health risk for both human (0.1 mg/kg) and animal (0.5 mg/kg) at levels that are not phytotoxic to plants (Peralta-Videa et al. 2009; Yurdakök 2015).

The data from this research showed that samples testing positive for Cd were 75% (0.09–1.12 mg/kg) and 34% (0.1–0.7 mg/kg) in the central and north, respectively. Furthermore, 27.7% and 5.5% of samples testing positive in central and northern region, respectively, were above the EU permissible limit. In two separate studies, Cd levels in poultry feed and ingredients from Pakistan and Bulgaria ranged from 0.11 to 1.41 mg/kg and 0 to 1.8 mg/kg, respectively (Alexieva et al. 2007; Imran et al. 2014). Although the intake of Cd by plants is generally below 1 mg kg<sup>-1</sup> of dry matter, cadmium contents higher than 10 mg kg<sup>-1</sup> have been reported in crops grown near industrial areas and in farm produce after application of cadmium-enriched fertilizers (Fink-Gremmels 2012; Wolf and Cappai 2020). Cd toxicity has been linked with nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity and negative reproductive effects (EFSA (European Food Safety Authority), 2004; EFSA (European Food Safety Authority), 2009).

For several decades, Cr was considered an essential nutrient; however, recently, this position has changed as the beneficial effects from Cr occur at high pharmacologically relevant doses, in excess of nutritionally relevant doses. As a result, the EFSA has recently determined that no evidence exists on chromium being a basic heavy metal for humans or animals (Vincent 2017). The data on Cr content in feed samples in this study were found to be at very low levels, insufficient to present health hazards. The highest concentration

recorded was observed in maize samples from central region (1.80 mg/kg). The chromium content in 12 different commercial poultry feeds (starter feed, grower feed, and finisher feed) from Bangladesh was analyzed, and the levels in 10 of the 12 feed samples were less than 0.03 mg/kg (Ahmed et al. 2017). In another study, Cr content in poultry feed samples from Al-Qassim region, Saudi Arabia, ranged from 0.14 to 1.82 mg/kg (Alkhalaf et al. 2010). On the contrary, Tao et al. (2020) found higher Cr content (0.13–93.04 mg/kg) in poultry feed from Hubei province, China. The maximum acceptable limits for Cr concentrations in food range from 0.1 to 0.5 mg/kg for human (Alkhalaf et al. 2010). Cr toxicity increases reactive oxygen species damaging proteins and DNA. Its severity is dependent on the type of Cr; with Cr (VI) regarded as being carcinogenic and causing problems in the internal organs and the circulatory system (Dai et al. 2016).

Although Cu is a required element for various enzymatic activities and different body functions (NRC 1994), it is also known to be equally toxic and gains access to food constituents from environmental contamination, food processing or through the soil during mineralization by crops (Mahesar et al. 2010). In this study, the concentration of Cu ranged from 0.50 to 17.00 mg/kg and 3.0 to 24.70 mg/kg in feed samples from the north and central, respectively. The highest concentration of Cu (24.70 mg/kg) was seen in layers mash feed from the central, while lowest levels were observed in maize samples from both regions. The range of Cu concentration found in this study was less than 19.2–36.1 mg/kg (Nicholson et al. 1999), 12.3–65.8 mg/kg (Mahesar et al. 2010) and 3.8–198.7 mg/kg (Wang et al. 2013) mentioned in the literature. The maximum permissible limit for copper in animal feed is 40 mg/kg (NRC 2005), and elevated concentrations of Cu in the human body provoke nausea, jaundice, diarrhoea, severe colic, liver and renal problems, as well as anaemia and Wilson's disease (Elsharawy and Elsharawy 2015; Ogwok et al. 2014).

Comparing the levels of heavy metal contamination between regions showed generally higher levels of toxic elements in the central and this occurrence could be probably due to the trace oil exploration activities that regularly pollutes the soils in the region. In addition, illegal artisanal heavy metal mining activities, use of leaded petrol and the dumping of electronic wastes have also been reported to contribute to environmental heavy metal contamination, poultry toxicity and human poisoning in Nigeria (Dooyema et al. 2012; Rabiu et al. 2019; Oladipo et al. 2020; Pona et al. 2021). Iftikhar et al. (2014) analysed the concentrations of heavy metals in milk samples from urban and rural areas in Pakistan and observed that samples from urban areas had higher levels of the toxic metals. Similarly, Cu levels in milk samples were higher in active mining areas with heavy





metal-rich soils compared to other regions in Iran (Shahbazi et al. 2016).

## Conclusion

The occurrence of AF and heavy metals in samples of maize, soybean meal, broiler finisher and layers mash was determined in two regions of Delta State, Nigeria. The result obtained from the study showed that AF contamination for all feed samples apart from maize in the northern region was marginally lower than permitted levels (20 µg/kg). On the other hand, AF contamination of samples from the central region was above-permitted levels, with soybean meal having the highest incidence of AF contamination. The data on heavy metals showed that while concentrations of Cd, Cr and Cu were generally below permissible levels, the average concentration of Pb in broiler finisher and layers mash was above-permitted levels, which can induce toxic effects in poultry birds. In order to ascertain the route of Pb contamination in these feeds, it may be necessary to analyse feed component materials separately.

Taken together, there is the need for constant monitoring of AF and Pb levels in poultry feeds and ingredients in these regions to ensure strict compliance with international standards and regulations. Furthermore, employing practical and cost-effective intervention measures for controlling AF and Pb contamination in developing countries will help reduce the health and economic risks associated with their occurrence.

**Author contributions** I.I designed the experiments and wrote the first draft of the manuscript. C. I., P. I. and W.A conducted the experiments under the supervision of I. I. O. O analyzed the data for the manuscript. I. I., U. A. E and J. O. A contributed to writing and preparation of the final manuscript. All authors read and approved the final manuscript.

## Declarations

**Conflict of interest** The authors have no conflicts of interest to declare that are relevant to the content of this article.

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