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Concentration and health risk assessment of toxic metals in giblets of free-range chickens in Lokoja, Nigeria

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ABSTRACT

Background: Potentially toxic metals present in food and food products are a threat to humans. To monitor the quality of giblets consumed and to evaluate the risk to the public's health, this study aimed to determine the residual concentrations of heavy metals (Al, As, Cd, Cr, Mn, Ni, Pb and Zn) in the gizzard, kidney, and liver of free-range chickens.
Methods: The mean concentration of metals in the giblets was determined using Inductively Coupled Plasma Optical Emission Spectrometry. The mean was used to assess the cancer risk, hazard index, total hazard quotient, and estimated daily intake of the metals under investigation.
Results: Metal concentrations ranged from 17.6 - 105.6 (Al), nd to 3.15 (As), nd - 4.38 (Cr), 0.26 - 73.6 (Mn), nd - 7.21(Ni), nd - 6.67 (Pb), and 8.42 - 63.3 (Zn) mg kg^{-1}. The mean concentrations measured exceeded JECFA's Maximum Allowable Concentrations limit except for Al and Cd. Cd was below the detection limit. The estimated daily intake of the toxic metals As, Cr, Ni, and Pb exceeded the threshold limit. The metals' calculated Target Hazard Quotient and Hazard Index values were ⟨ 1. The percentage contribution of Pb to the HI value was the highest. The HI was 66.9% and 65.4% in adults and children, respectively. The HI sequence through the consumption of giblets in adults is Pb ⟩ As > Cr > Ni > Zn > Al > Mn. The corresponding sequence in children is Pb > As > Cr > Mn > Ni, Zn > Al. The carcinogenic risk of As, Cr, Ni, and Pb exceeded the threshold limit, indicating a potential cancer risk through consumption.
Conclusion: Free-range chickens are not selective in picking their food in the environment hence strict regime of proper disposal of waste products containing metals into the environment should be advocated and followed.

Introduction

An important food-producing industry in the world is poultry farming. It serves as the primary source of protein for millions of people globally [1]. The viable poultry business is well-developed worldwide. It is an utmost supplier of animal protein as an excellent source of essential amino acids, vitamins, and minerals for human consumption in the form of meat and eggs, in the commercial chicken industry [2,3]. Global egg production has increased significantly and according to a report it reached 83 t in 2019, up 63% from 2000, poultry meat accounted for around 40 % of global meat output in 2019, underscoring its importance as the world's most produced meat [1].

Chicken meat is trendy, and it can be prepared in different forms. It can be prepared with stews, fast food such as burgers, luncheon meat, frankfurters, and processed food. People consume chicken giblets such as liver and gizzard as part of their diet, mostly in developing countries [2,4]. In some cultures, in south-south Nigeria, like the Urhobos in Delta State, the gizzard is so significant that it is reserved for the head of the family [5]. The choice of chicken is due to its low-fat content and richness in proteins of high biological value, polyunsaturated fatty acids (PUFAs), minerals (e.g., Fe, Se, and Zn), and vitamins (e.g., A, B2, B6, and B12) [4] and its high meat production, low cost, and low cooking loss [3]. However, chicken products may cause worries due to contamination with potentially toxic metals (PTMs) that occur naturally or anthropogenically in air, soil, water, and poultry diets [2,3]. The spread of metals has been hastened by growing anthropogenic activity patterns such as industrial waste, mining, chemical pesticides and enrichers, uncontrolled sewage discharge, and extensive groundwater

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irrigation. Some metals are toxic and pose serious threats to animals and humans when ingested through the food chain [1,6].

Metals, especially Cd and Pb, are undegradable and poisonous. Once consumed in the food chain, they are absorbed and will mostly disperse across several tissues, mainly the liver and kidneys [1]. They can also accumulate in the human body via bioaccumulation. The buildup of a high level of metals activates a variety of deadly symptoms, such as hepatic–renal dysfunction and reproductive issues [7]. Cd in the human body causes hypertension, kidney dysfunction, pulmonary and hepatocellular tissue damage, and lung and liver damage [8]. It builds up in the liver after an acute exposure and is connected to certain hepatic dysfunctions. Cd changes the cellular redox balance, causing oxidative stress and hepatocellular damage. Cadmium-induced hepatotoxicity, whether acute or chronic, can result in liver failure and elevate the likelihood of developing cancer [7]. Exposure to cadmium can harm the kidneys, induce bone loss, and raise the risk of cancer [9,10]. Pb is a neurotoxin that can impair metabolism and negatively affect the neurological, gastrointestinal, hemopoiesis, and renal systems [1,11]. Pb exposure can block heme synthesis and harm the brain and kidney systems [1]. Its poisoning surges neurologic and psychiatric morbidity. Early indications of Pb neurotoxicity include headaches, impaired concentration, and irritability [11]. Even modest levels of lead exposure can cause behavioural issues, developmental delays, and cognitive impairment in kids [9]. Pb can deactivate antibodies, reducing poultry's ability to fend against viral diseases. Additionally, Pb poisoning decreases lysosome function and influences polymorphonuclear leukocyte phagocytic activity. Lastly, Pb inhibits the functions of numerous antioxidant defences; low antioxidant levels can harm multiple organ systems, such as the kidneys, liver, neurological system, and reproductive system. Poultry has also been demonstrated to die from Pb toxicity in extreme circumstances [1,12,13].

Nickel has a wide range of industrial applications and is a naturally occurring element. It is released into the atmosphere by man-made and natural sources. When ingested, it has numerous negative impacts on humans, including allergies, lung and nasal cancer, kidney, and cardiovascular disorders [14,7]. The nervous system is one of the main target organs for Ni toxicity; it can be accumulated in the brain. Allergy to nickel and metals is caused by the materials used in our daily lives; therefore, the chances of triggering the onset of allergic reactions are high. This metal can cause an allergy that manifests as contact dermatitis, headaches, and gastrointestinal and respiratory manifestations [14]. The accumulation of Ni and its compounds in humans through chronic exposure may lead to a diversity of adverse effects on human health, like lung fibrosis, cancer of the respiratory tract, and kidney and cardiovascular diseases [14,15]. Numerous health issues, such as skin lesions, cardiovascular illness, and an elevated risk of cancer, can result from exposure to arsenic [9].

The toxicity of these metals is partially due to their tendency to build up in living tissues, a phenomenon called bioaccumulation. Metal bioaccumulation happens in all living organisms, including food animals and humans, due to exposure to metals in food and the environment. The adverse impacts of these metals can include harm to the central and peripheral nervous systems, the gastrointestinal and reproductive systems, liver and kidney toxicity, weakened immune systems, and the development of cancer. One of the most significant global environmental issues is the presence of PTMs. The increasing health risks posed to humans through the food chain and occupational and environmental exposure lead to several serious diseases [3]. Poultry frequently contract heavy metal contamination from contaminated water, sewage water, poultry feed, industrial waste, and aerial spraying in poultry breeding areas [2]. Due to widespread and increasing environmental pollution by PTMs of anthropogenic origin, investigators recommend the continuous collection of data as possible on various species of mammals, especially those living near humans [8]. Determining potentially toxic metals accumulated in free-range chicken may serve as a bioindicator of the concentration of metals in the environment and their entrance into the food chain. Metals enter a variety of food sources from the environment and are passed to animals and humans through the food chain [1,16]. The kidneys and liver are vital for detoxification and the excretion of hazardous substances in animals and humans. These organs are the most harmed when toxic metals are consumed from contaminated feeds and water [1]. Some metals are highly toxic even at low concentrations, so monitoring their concentrations in giblets of free-range chicken is critical for food safety and human health. This study aims to: (i) determine the concentration of Al, As Cd, Cr, Mn, Ni, Pb, and Zn in giblets of free-range chicken in Lokoja using the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) technique, and (ii) to estimate the risks of both cancer and non-cancer in the local population due to the associated metal exposures from their intake. It is vital to assess the likely health risk that the consumption of poultry edibles poses to the local population.

Material and methods

Sample collection

In Lokoja, Nigeria, a combined total of 50 free-range chickens were purchased and slaughtered. The chickens were purchased from individuals in five different areas within Lokoja City (Fig. 1) Each bird was de-feathered and washed with distilled water. The gizzard, liver, and kidney were extracted from the body, placed in a labelled zip-lock bag, and kept in a fridge before analysis.

Calibration of instrument and operation

The multi-element calibration standard PE-MECAL3-ASL-1 was the reference material used to prepare the calibration and quality control (QC) solutions. Ultrapure Merck Lichrosolv water was used to dilute the standards and QC solutions. A high purity 2 % v/v concentrated nitric acid was used to stabilize the solutions. Working standard concentration ranges from the multi-element stock standard were created using the serial dilution approach. Each sample code and the procedure parameters were programmed into a spreadsheet created by the ICP-OES expert software (Table 1).

The coil was first torched with argon gas, and plasma was produced by delivering a high-frequency electric current to the work coil at the tip of the torch tube. The high-frequency current in the torch tube created an electromagnetic field that was used to ionize argon gas, resulting in plasma. This plasma's high electron density and temperature (10,000 K) provided the energy used in the sample's excitation-emission process. In an atomised state, solution samples were fed into the plasma through the small tube in the centre of the torch tube [17,18].

Sample preparation and quality assurance

Each sample was allowed to thaw and macerated using a stainless-steel knife and 2.0 g was weighed and used. Using a digestion mixture of 60 ml of 65 % HNO_3 acid and 40 ml of 70 % $HClO_4$ (Sigma-Aldrich Munich, Germany) in a Pyrex flask with vigorous mixing, the digestion process was carried out following Tsoumbaris & Tsoukali-Papadopoulou method [19]. The processed sample was passed through Whatman filter paper No 42 (Sigma-Aldrich). The filtered liquids were collected and stored in a plastic bottle and used to measure the concentrations of Al, As, Cd, Cr, Mn, Ni, and Pb using ICP-OES (Agilent 720-ES, New York). Samples and blank solutions were analysed together. The same reagent was used to prepare the blank solutions, but no samples were included. Every chemical and salt that was used was of Analar R grade. Blank solutions were evaluated alongside the samples to account for errors caused by the reagents used. A blank digestion was also made for every 10 samples. Every analysis was completed in triplicate, and the results of their averages were shared. Both recovery studies and appropriate quality control procedures were followed [20]. The glassware was

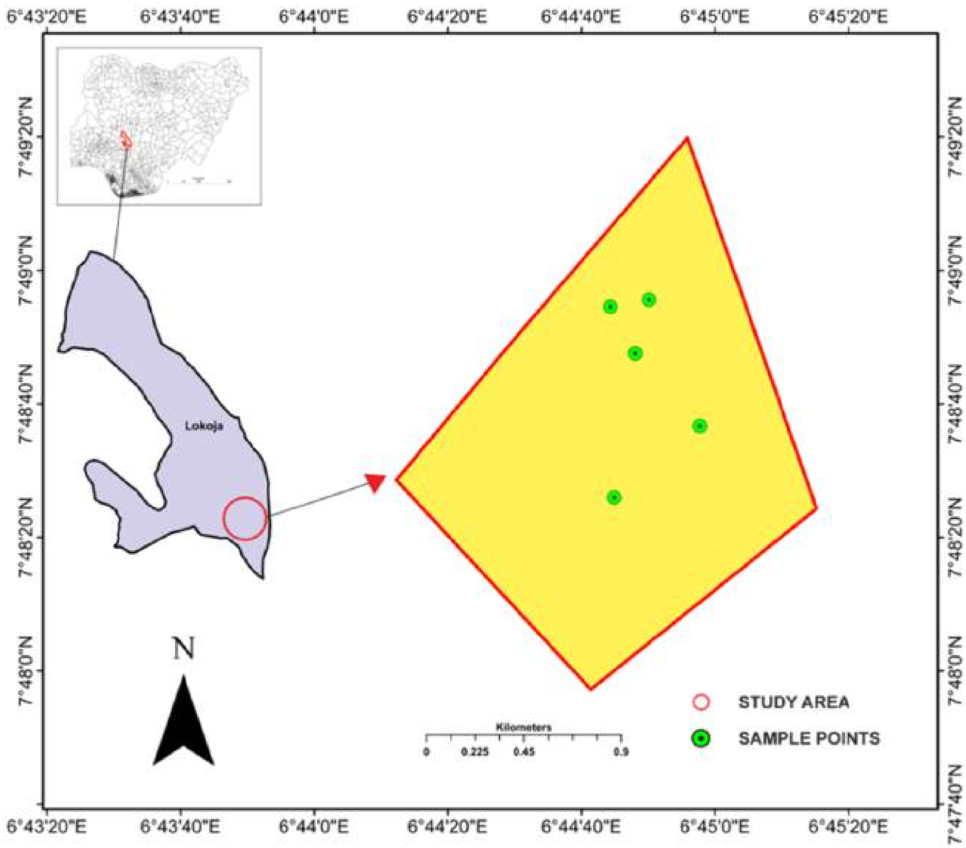

Fig. 1. Map of the study area.

Table 1
Optimization parameters of ICP-OES used in this study.

Type of detector	Charged couple devices (CCD)
Power	1 kw
Plasma gas flow	15 L min-1
Auxiliary gas flow	1.5 L min-1
Spray chamber type	Glass cyclonic
Torch	Standard axial torch
Nebulizer type	Sea Spray
Nebulizergas pressure	220 kPa
Pump speed	15 rpm
Sample uptake	30 s
Replicate read time	30 s
Number of replicates	2
Sample delay time	20 s
Stabilization	15 s
Rinse time	10 s
Fast pump	On
Wavelength (nm)	Al (396.152), As (188), Cd (214.439), Co (238.892), Ni (231.604), Pb (220.353), Mn (257.610), Cr (267.716), Zn (213.857)

meticulously cleaned with Teepol, rinsed with distilled water, immersed in 2 M HNO_3 acid for a whole day, and then washed with deionised water.

Recovery studies

As a test of analytical quality assurance, standard addition by spiking and homogenising previously analysed samples and concentrations known with varying amounts of standard metals solutions was conducted using triplicate analysis to obtain recovery of the method and to assess the accuracy and precision of the procedure. The unspike and spiked samples were analysed similarly, and each metal's percentage recovery was calculated using Eq. (1).

$$\% \, Recovery = \frac{S_{SP} - U_{unspk}}{Q} \tag{1}$$

The concentrations of spiked and unspiked samples are denoted as S_{sp} and U_{unspk}, respectively, with Q representing the spiking concentration [21].

The metals recovered average between 95.5 % and 99.6 % of their original value (see supplemental Table S1). Limit of detection (LOD) calculations followed Shrivastava and Gupta's instructions [22] and for Al, Cd, Cr, Ni, and Pb, the respective detection limits are 0.00197, 0.00011, 0.00125, 0.00072, and 0.00240 mg kg^{-1} respectively. It was estimated using the analyte concentration equal to the sample mean blank value plus three standard deviations of the blank values as in Equ. (2).

$$LOD = X_{BI} + 3S_{B1} \tag{2}$$

where X_{B1} represents the blank's mean concentration and S_{B1} represents its standard deviation.

Statistical analysis

The collected data were statistically analysed to determine the mean and standard deviation. Before analysis, they underwent tests for homogeneity of variance and normality. A one-way analysis of variance (ANOVA) was conducted at a significance level of 5 % to determine if there were significant differences in heavy metal levels between giblets. Pearson correlation, Principal Component Analysis (PCA), and Cluster Analysis (CA) were conducted to get comprehensive data regarding the distribution of PTMs and their similarities and differences in the samples. OriginPro 2022 was used for all statistical computations, graphs, and doughnut charts, while Excel calculated the mean, standard deviation, and range. To clarify whether the possible sources of metal contamination are anthropogenic or natural a variety of multivariate data analysis techniques PCA and HCA were used [20,23].

Health risk assessment

The health risk index (HRI) and estimated daily intake (EDI) were calculated to assess the probabilistic dietary risk for individuals (adults and children).

Estimated daily intake (EDI)

The metals estimated daily intake (EDI) was calculated using the following formula in Eq. (3) [24]:

$$EDI = \frac{C_m \times I_{ntr}}{B_{wt}} \quad (3)$$

Where: B_{wt} is the average body weight of Nigerians; I_{ntr} is the intake rate (g/giblets that Nigerians consume daily. Nigerian population, which is considered 32.7 kg per person/day) [24], which in Nigeria equals 1.16 kg (= 3.18 g/day) in 2020 was obtained from an online source Helgilibrary.com as contained in FAOSTA [25]. The approximate Nigerian population consumption per day is 0.32 g of chicken giblets in chicken meat is <10 % and C_m is the mean concentration of PTMs in various chicken tissues (mg kg^{-1}). Therefore, the ingestion rate is equivalent to the nominal amount of 0.32 g (0.32×10^{-3} mg kg^{-1}) for children and 70 kg for adults [6,24].

Target hazard quotient (THQ) and hazard index (HI)

The THQ defines the non-carcinogenic health risk linked with the chemical and denotes a ratio between the exposure and the reference dose (RfD). A tolerable reference dose (RfD) is the maximum level of toxic substances that do not harmfully affect human health [4]. Oral values of RfD for As, Al, Cr, Mn, Ni, Pb and Zn are 0.0003, 1.0, 0.003, 0.14, 0.02, 0.0035, and 0.4 mg kg^{-1} day^{-1}, respectively [26,27]. The THQ was calculated according to the USEPA Region III Risk-based Concentration Table [28] and by Chowdhury and Alam [29] as in Eq. (4).

$$THQ = \frac{C_m \times Ed \times I_{ntr} \times Ef}{B_{wt} \times A_t \times RfD} \times 10^{-3} \quad (4)$$

Where C_m is the metal concentration in the gizzard, kidney, and liver (mg/kg, w/w), Ed is the exposure duration (70 years for non-cancer risk), I_{nrt} is the food consumption rate (g/person/day), Ef is the exposure frequency (200 days/year), Bt is the average body weight for adult (70 kg) and children (32.7 kg), A_t is the average exposure time for non-carcinogens (Ef × Ed) (200 days/year for 70 years (i.e. $A_t = 14{,}000$ days) taking into account that chicken giblets are typically consumed four days a week in the area from the local information obtained, and RfD oral reference dose.

The HI is used to evaluate the total risk of non-cancerous health issues related to the intake of various metals [19].

Eq. (4a)

$$HI = THQ_{Al} + THQ_{As} + THQ_{Cd} + THQ_{Cr} + THQ_{Mn} + THQ_{Ni} + THQ_{Pb} + THQ_{Co} \quad (4a)$$

When THQ and HI values are > 1, it indicates a high risk and could have adverse effects on the health of humans. However, THQ and HI values < 1 are deemed safe [28,17].

Cancer risk (CR)

CR was calculated as in Eq. (5):

$$CR = EDI \times CSFo \quad (5)$$

Where EDI is the estimated daily intake (mg/ kg/ day), CSFo is the cancer slope factor for As (1.5), Pb (0.0085), Cd (0.38), Cr (0.5), and Ni (1.7) (mg/kg/day^{-1}) [30]. CSF for Al and Mn is unavailable, hence the inability to calculate their carcinogenic risk. USEPA has recommended that CR below 1.0×10^{-6} is insignificant and may not have any cancer risk effect while CR above 1.0×10^{-4} is substantial and can contribute to cancer risk [27].

Results and discussion

Concentration of metals in free-range chicken giblets

The concentrations of Al, As, Cr, Mn, Ni, Pb, and Zn found in free-range chicken giblets are shown in Table 2. The concentration (mg kg^{-1}) of metal on a dry weight basis ranged from 17.6 to 105.6(Al), nd to 3.15 (As), nd to 4.38(Cr), 0.26 to 73.6(Mn), nd to 7.21(Ni), nd to 6.67 (Pb), and 8.42 to 63.3(Zn). The mean concentration is in the order Al (55 ±40 to 99±78) > Zn (24±12 to 42±19) > Pb (3.5 ± 1.5 to 5.2 ± 2.4) > Cr (2.55±0.32 to 5.2 ± 1.7) > Mn (1.6 ± 1.9 to 4.6 ± 3.1) > Ni (1.82 ±0.31 to 2.5 ± 1.5), As (1.9 ± 1.2 to 2.2 ± 2.2) mg kg^{-1}. The variation reflects the various tissues' capacities for metal absorption and accumulation in food and the environment [31] and the foraging diet of free-range chicken [32]. Cd is a common toxic metal often transmitted through soil-water-animal routes [29] and was below the detection limit in the giblets of chicken determined.

Al is several-fold higher than the other metals investigated. It is a commonly found element that humans are easily exposed to. Consuming food that contains Al is one way of being exposed to it, as it is absorbed through the lining of the small intestine. Human exposure to Al is unavoidable and possibly fathomless. Remnants of Al compounds can be found in air, food, drinking water, deodorants, buildings, cosmetics, packaging, medicine, many appliances and equipment, aerospace engineering and transportation industries [33]. A research report states that the free Al metal cation, Al^{3+}(aq), is highly biologically reactive and biologically available Al is essentially toxic [34]. The effects of Al poisoning are complex and multifaceted, involving changes in protein synthesis, nucleic acid function, and cell membrane permeability; disruption or inhibition of enzyme activities; prevention of DNA repair; modification of the stability of DNA organization; inhibition of the activity of the protein phosphatase 2A (PP2A); increased production of reactive oxygen species (ROS) and oxidative stress; reduction in the activity of antioxidant enzymes; alteration of cellular iron homeostasis;

Table 2
Concentrations of metals in free-range chicken giblets ($n = 50$).

Metals	Mean ± SD (mg kg^{-1})			Range	MAC
	Gizzard	Kidney	Liver		
Al	99±78	55±40	79±48	17.6 - 105.6	200 mg kg^{-1} [a]
As	1.9 ± 1.2	nd	2.2 ± 2.2	nd to 3.15	0.1[b]
Cd	nd	nd	nd	nd	
Cr	2.55 ±0.32	2.91 ±0.68	5.2 ± 1.7	nd - 4.38	1.0[c]
Mn	4.6 ± 3.1	1.6 ± 1.9	1.7 ± 3.3	0.26 - 73.6	–
Ni	2.5 ± 1.5	1.82 ±0.31	2.48 ±0.85	nd - 7.21	0.5[c]
Pb	3.5 ± 1.5	5.2 ± 2.4	4.2 ± 2.0	nd - 6.67	0.1[c]
Zn	24±12	31±5.9	42±19	8.42 - 63.3	–

nd: not detected.
[a] Codex, 1995.
[b] JECFA 2005.
[c] Demirezen and Uruç, 2006.

and modification of the NF-kB, p53, and JNK pathway resulting in apoptosis [33]. Al poisoning has been identified as a major etiological factor in Alzheimer's disease, among other pathological diseases [35]. The concentration of Al was highest in the gizzard with a mean of 99±78 mg kg^{-1}. Corresponding values in the liver and kidney were 79±48 and 55±40, respectively. The results for Al were lower than the reported values of 8.44 and 16.44 g/g in the liver of chicken [36], a range of 5.873 to 14.005 g/g in the liver of six brands of poultry birds [37]. Although the concentrations in this study were higher, they are within the 200 mg kg $^{-1}$ maximum tolerable level (MPL) according to the Codex Alimentarium Commission [38].

Arsenic (As) exposure has been linked to the cause of acute sore throat, diarrhoea, vomiting, anorexia and arrhythmia [39]. The highest mean concentration of As was found in the liver (2.2 ± 2.2 mg kg^{-1}), and the lowest was 1.9 ± 1.2 mg kg $^{-1}$ in the gizzard but was not detected in the kidney. This study's concentrations are higher than 0.318±0.071(gizzard), 0.233±0.025 (liver) and 0.196±0.015 (kidney) [40], 0.263±0.048 (liver), 0.203±0.073 (gizzard) and 0.171±0.015 (kidney) [41] in southeast Nigeria, 0.0471±0.026 (gizzard), 0.061 ±0.073 (kidney), and 0.069±0.091 (liver) in southern Nigeria [25,32] 0.011±0.002 (liver) and 0.007±0.001(gizzard) in Tehran, Iran [4], 0.01 in liver and gizzard [31] in Malaysia, 0.09±0.007 (kidney), 0.06±0.004 (liver) and 0.10±0.008 (gizzard) [35,42] in Turkey. The results of this investigation are consistent with those of previous investigations [42,43,], which found that the liver had the highest As concentration. In another study, the gizzard had the highest As contamination [42]. The reason for the gizzard's high metal content is that it serves as the main organ for processing and storing feed [4]. The present work indicates that the mean value in the gizzard is below the permissible limit of 2 μg/g while the concentration in the liver is above the threshold limit [44].

Although Cr is considered an essential element in minimal amounts, high concentrations of it have been linked to cancer, toxicity, and adverse effects on the kidneys, liver, bones, and teeth [26]. Moreover, the absorption of Cr in the lungs and gastrointestinal tract is responsible for its toxicity [38]. In this study, Cr concentrations ranged from 2.55 ±0.32 mg kg^{-1} (gizzard) to 5.2 ± 1.7 mg kg^{-1} (liver) which are similar to the values reported by other studies [44,26] but higher than these studies [32,40,41] in Nigeria, [31] in Malaysia, [42] in Turkey, and [46] in Egypt. The mean Cr content in the giblets was higher than the maximum permissible limit of 2.3 mg kg^{-1} for foodstuffs [47].

Many things can release, Ni into the environment, such as batteries, automobiles, electroplating procedures, steel production, fertilisers, and mining [4]. According to Genchi et al., it is distributed extensively in the environment [14]. Exposure to Ni can cause numerous adverse health effects, including allergies, kidney and cardiovascular disorders, lung fibrosis, and lung and nasal cancer. They opined that Ni toxicity is the cause of mitochondrial dysfunctions and oxidative stress [14]. A high concentration of Ni is needed to enter the body via food to cause toxicity, hence the risk of Ni poisoning via food is less than that of inhalation [4]. The mean concentration of Ni was highest in the gizzard (2.5 ± 1.5 mg kg^{-1}) followed by the liver (2.48±0.85 mg kg^{-1}) and the least kidney (1.82±0.31 mg kg^{-1}). The concentrations exceed the maximum allowable concentration (MAC) of 0.5 as specified by JECFA [48]. These concentrations are similar to 1.82 mg kg^{-1} in meat and 1.04 mg kg^{-1} in the gizzard in Tehran, Iran [4], 1.76±0.39 05 mg kg^{-1} (gizzard) East of Iran [26], 1.909±0.96 mg kg^{-1} in the liver and 1.839±0.43 mg kg^{-1} in the gizzard in Malaysia [45]. This study's results are lower than 8.58 ±0.39 mg kg $^{-1}$ (brain), 7.39±0.22 mg kg^{-1} (muscles), and 6.34±0.1 mg kg^{-1} (liver) in Bangladesh [29]. This study results are, however, higher than 0.018±0.009 (liver), 0.020±0.004 (kidney, and 0.049 ±0.037 (gizzard) in Benin City, Nigeria [32].

When food containing Pb is consumed, it effortlessly passes the blood-brain barrier, accumulates in the brain, and causes damage to the central nervous system [29]. The level of exposure determines the negative consequences of Pb. It can range from minor physiological or biochemical abnormalities to significant pathologic illnesses, resulting in various organ damage, systems harmed, and their functions altered. Pb, for instance, inhibits the functions of numerous antioxidants, resulting in reduced levels of antioxidants. As a result, this can cause damage to various organ systems such as the neurological system, liver, kidneys, and reproductive system [1]. The concentration (mg kg^{-1}) of Pb was highest in the kidney (5.2 ± 2.4), followed by the liver (4.2 ± 2.0) and least in the gizzard (3.5 ± 1.5). The concentrations exceeded the MAC of 0.1 mg kg^{-1} [48]. These results are consistent with Demirezen and Uruc's findings that the liver and kidney are the main sites where metals are found in animals because of exposure and the bodies' natural detoxification processes [49]. Similar results have been reported in Pakistan [50,51], Bangladesh [29] and Abuja, Nigeria [52]. This study's concentrations, however, were higher than the results reported in Benin City, South-South Nigeria [32], in South Eastern Nigeria [40], in Tehran, Iran [4], East of Iran [26], in Turkey [35,42], and in Selangor Malaysia [48].

Mn is an essential element for animals and plants. Its deficiency results in severe skeletal and reproductive abnormalities in mammals [32, 53]. However, excess of it is toxic. Its toxicity in the brain is said to cause Parkinson-type syndrome [32]. The Institute of Medicine's recommendation is that the daily intake of Mn from water, food, and dietary supplements should not be above 11 mg in a day. The intake of Mn in the giblets is below the tolerable limits. The mean concentration of Mn was highest in the gizzard (4.6 ± 3.1 mg kg^{-1}) and lowest in the kidney (1.6 ± 1.9 mg kg^{-1}). The Mn concentration in the liver and kidney in this study is lower than 3.607±1.198 (liver) and 2.468±0.565 (kidney) in a previous study in Benin City, Nigeria [32], 12.70 ± 0.46 μg-g^{-1} in Abuja, Nigeria [51], 2.51 ± 0.178 mg kg^{-1} in Turkey [42]. However, the Mn concentration in the gizzard is higher than 0.547±0.204 mg kg^{-1}[25], 0.05 ± 0.003 [42],

The majority of human metabolic processes are known to require Zn. It is needed to maintain vital biological functions such as gene expression, genetic stability, DNA repair and programmed cell death [54]. A Zn deficit can result in immunological problems, growth retardation, appetite loss, and skin changes, while high levels of it can cause pancreatitis, muscle pain, anaemia, and acute renal failure [42,49,54]. In this study, the mean concentration of Zn was highest in the liver (42 ±19 mg kg^{-1}) followed by the kidney (31±5.9) and the least in the gizzard (24±12 mg kg^{-1}). These concentrations are lower than 85.93 ±7.89 μg g^{-1} (gizzard) and 78.86±21.45 μg g^{-1} (liver) reported in Malaysia [38,45] but higher than 22.5 ± 2.1(liver) and 21.0 ± 1.9 (gizzard) in Turkey [42], 5.27±0.59 (liver) and 3.15±0.39 (gizzard) in Egypt [39], 1.20±0.79 (liver) and 1.00±0.51(gizzard) in Nigeria [33], 2.5 ± 0.4 (liver), in Malaysia [24], and 1.342 (liver) in Iraq [55]. Chicken giblets are a primary source of Zn metals necessary for growth, oxygen transfer in organisms, and the building of nutrients [31]. The recommended daily consumption of Zn is 15 mg for adult males, and for adult females, it is 12 mg [42].

Distribution of metals in free-range chicken giblets

The Pearson correlation coefficient is often used as a potential tool to evaluate the degree of the linear link between the pairs of variables by computing a summary index [56]. As a result, the metal-to-metal correlation data's significant Pearson product-moment correlation coefficients at the 99 % and 95 % confidence levels were evaluated (Fig. 2). A strong positive relationship exists between Cr and Zn (0.84), Mn and Zn (0.65), and Cr and Mn (0.56) at 99 %. A slight positive correlation was found between Ni and Zn (0.49) and Ni and Al (0.45). These positive relationships suggest a potential shared source, indicating that the chickens have picked food from a polluted environment. Ogbomida et al. have reported similar interactions between metals in chicken giblets in Benin City, south-south Nigeria [32] Nigeria. Free-range local chickens have no oral discrimination for food as they pick whatever they find edible in the environment [32].

The principal component analysis (PCA) on the giblets of free-range

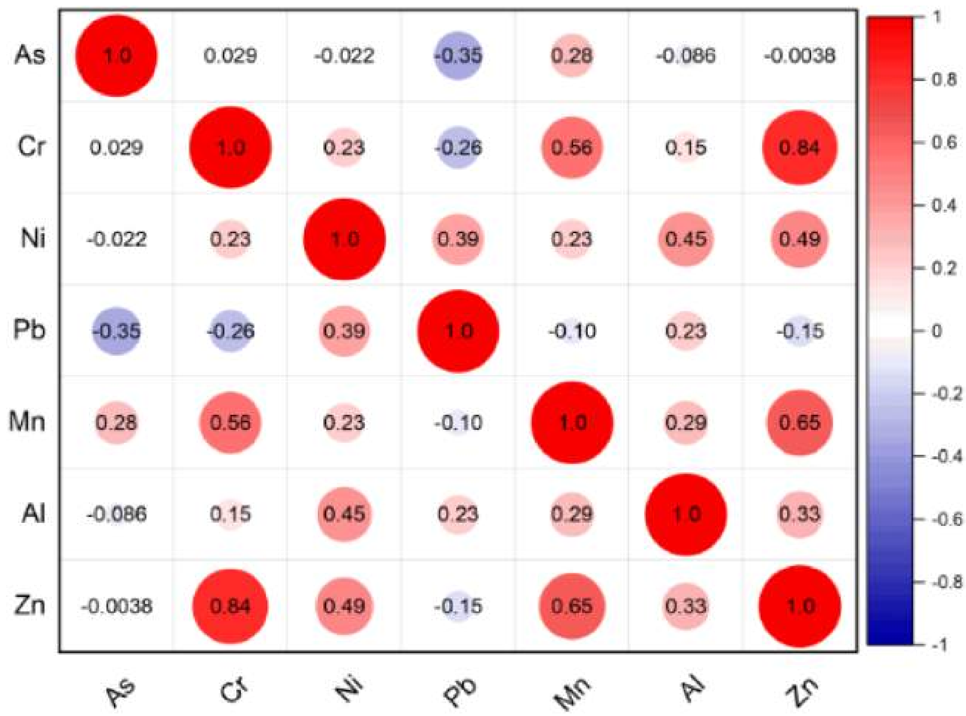

Fig. 2. Correlation between the heavy metals in offal.

chicken data employing varimax-normalized rotation was performed to spot the relationship of cluster variables in simple ways. The multivariate structure of the data, a potential pattern, and the probable source of heavy metal contamination were all identified using PCA [57,58]. The calculated factor loadings, cumulative percentage of variation, and percentages of total variance explained by each component are displayed in Table 3, while Fig. 3 display the loading plots for the three components for a better understanding of the relationship among metals. Factor loading establishes the connections between each factor and the variables. The results showed that three eigenvalues greater than 1, accounted for about 74.98 % of the total variance. The first component (PC1) accounted for 34.97 % of the variance overall and had the highest loadings for Zn (0.551), Cr (0.460), Ni (0.434), Al (0.406), and Mn (0.325), indicating that these metals are from a common source. The loading values were large, positive, and close to each other. PC2 described 24.91 % of the total variance with high loading for Pb (0.655). The high Zn, Cr, Ni, Al, and Mn levels in component one and the high Pb loading in component two indicate that these metals are generated from a common source of long-term anthropogenic activities such as municipal solid waste, which may have significantly contaminated the environment with toxic metals or intake of water contaminated with metals [59,32]. The third component (PC3) accounted for 15.10 % of the variance, revealing a high positive factor loading of 0.566 (Ni) and

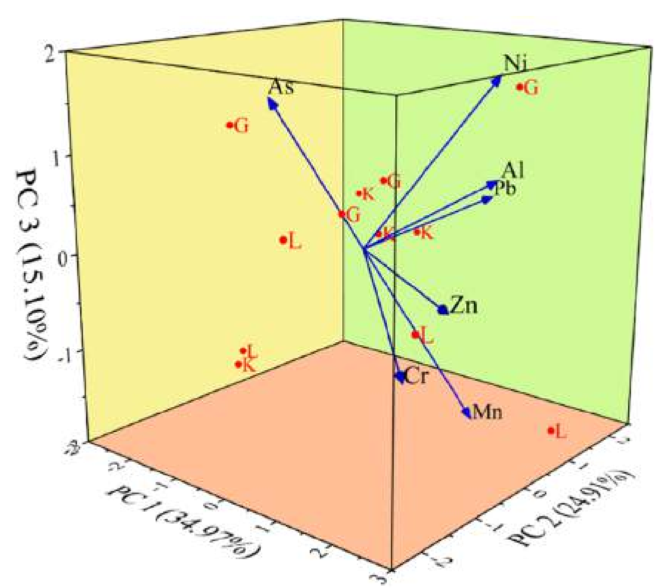

Fig. 3. Loading plot of the three components of the data.

0.505 (As) indicating their similarity [60,61]. One of the leading causes of inorganic chemical poisoning in farm animals and a major hazard to human health is arsenic. These potentially toxic metals are important human-caused environmental contaminants, particularly from industry, improper disposal of lead-acid batteries, metal items (such as pipes and solder), and inadequate urban waste management systems prevalent in developing nations. Electronic garbage, particularly used computers, cell phones, and other devices that contain dangerous elements like lead, cadmium, and nickel, is now being dumped in the environment. Over time, these electronic wastes add to the growing environmental problems by ending up at municipal garbage dump sites, which contain a mixture of combustible and non-combustible elements Ogbomida et al. [32]. A holistic waste disposal mechanism will be of importance.

Table 3
Principal component loadings (varimax-normalized) and communalities for heavy metals in samples ($n = 50$).

Heavy metals	PCI	PC2	PC3	Communalities
As	−0.095	−0.406	0.505	0.429
Al	0.406	0.228	0.244	0.276
Cr	0.460	−0.423	−0.239	0.448
Ni	0.434	0.173	0.566	0.538
Pb	0.133	0.655	0.091	0.447
Mn	0.325	0.222	−0.546	0.453
Zn	0.551	−0.310	−0.039	0.401
Eigenvalue	2.448	1.744	1.057	
% of the total variance	34.97	24.91	15.10	
Cumulative% of variance	34.97	59.88	74.98	

Hierarchical cluster analysis (HCA) of metals in giblets

Based on metal values, hierarchical correlational cluster analysis was used to assess the homogeneity and association of metals within the chicken giblets. The dendrogram (Fig. 4) shows the result of the HCA, where five clusters are displayed. Cluster 1 contains As, which demonstrates the clustering connection within the giblets. In cluster 2 are Al and Ni which form a subcluster with cluster 3 containing Pb, indicating similarity between these metals as they are perhaps from a common source [58]. Found in cluster 4 are Cr and Zn, which made a sub-cluster with Mn in cluster 5, a sign of their close relationship but mild industrial source [62]. The HCA results obtained, agree with the PCA analysis and are consistent with the interpretation.

Health risk assessment

Estimated daily intake (EDI)

The population's health risk assessment, shown in Fig. 5, was calculated using the EDI value for adults and children. The daily intake of metals determines how harmful they are to humans. According to the EDI values for metals Al, As, Cr, Mn, Ni, Pb and Zn for an adult person of 70 kg and children with 32.7 kg body weight showed that the gizzard of chicken had the highest EDI values in adult and liver in children. The order in an adult is gizzard > liver > kidney, while in children, liver > gizzard > kidney. The EDI of the consumption of gibbets (gizzard, kidney, and liver) by adults was in the order Al > Zn > Pb > Cr > Mn > Ni > As. Similarly, for children, it was Al > Zn > Ni > Pb > Cr > Mn > As. The value for Ni and Pb via the consumption of giblets was above the threshold limit for adults and children. Also, the value of Cr in liver consumption for children is high (Supplementary Materials Table S2). For children, the EDI showed that the consumption of the liver will most likely lead to health risks while in adults the consumption of the gizzard.

Target hazard quotient (THQ) and hazard index (HI)

The process of determining the possible health consequences of a given pollutant on people derived from one or more exposure pathways is known as risk assessment. The adult population's non-carcinogenic giblets consumption risks were evaluated using target hazard quotients (THQ). The dosage of the metal under investigation is divided by the reference dosage level for a metal that is similar to determine the THQ. If the ratio exceeds 1, population exposure may have adverse health consequences that are not carcinogenic [63,64]. The assessed THQ and HI values of the metals in this study are in Table 4. The THQ of the examined metals was lower than 1. Given that the THQ of the metals under investigation was <1, eating the chicken giblets does not pose an appreciable adverse risk to the health of children and adults. The impacts of all the elements are expressed using the HI, which enables one to assess the cumulative health risks of the metals. A consumer's risk or hazard is higher when the HI level is > 1, and one to ten suggests a moderate risk, while less than one indicates no danger [26]. HI of the consumption of free-range chicken giblets in adults and children was < 1, suggesting that eating the giblets will not expose consumers to any potential non-carcinogenic health issues. Although the HI value was <1, the results show that Pb was the highest contributor (Fig. 6). The percentage contribution of Pb to HI value was 66.9 % and 65.4 % in adults and children, respectively. The HI sequence through the consumption of the chicken giblets in adults is Pb > As > Cr > Ni > Zn > Al > Mn. The corresponding sequence in children is Pb > As > Cr > Mn > Ni, Zn > Al.

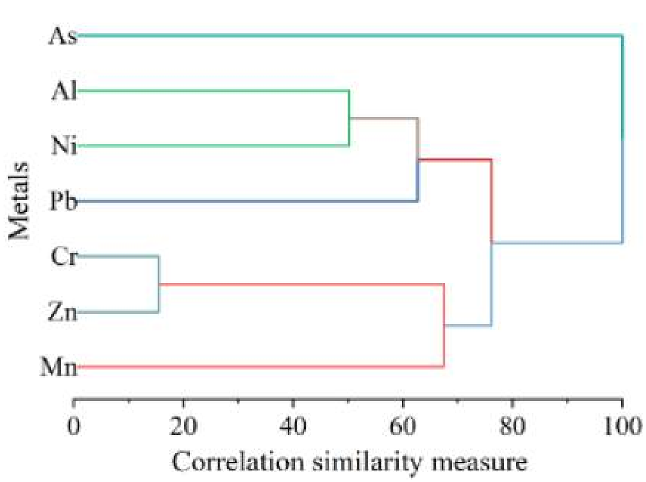

Fig. 4. Dendrogram showing clustering of the analyzed metals.

The carcinogenic risk (CR)

The risk of acquiring cancer was estimated using the concentrations of possible carcinogens (As, Cr, Ni, and Pb) in the giblets. The calculated CR values of metals are shown in Table 5.

The CR values from exposure of As ranged from 1.35E-02 to 1.50E-02 and 2.58E-02 to 3.30E-02, while for Cr, it was 6.00E-03 to 1.20E-02 and 1.25E-02 to 2.52E-02, for Ni it was 1.36E-02 to 1.87E-02 and 3.06E-02 to4.08E-02, and for Pb it was 1.36E-04 to 2.04E-04 and 2.89E-04 to 4.34E-04 for adults and children, respectively. TCR values below 1.0E-06 are considered insignificant, those above 1.0E-04 are unacceptable, and those falling between 1.0E-06 and 1.0E-04 are within an acceptable range [65]. The result revealed that the risk of As, Cr, Ni, and Pb due to the consumption of the giblets exceeded the acceptable threshold (CR > 1.00×10^{-4}). It is crucial to take into account the potential for free-range chicken giblets consumption to put people in the area at risk for cancer. Exposure to various metals has been linked to different types of cancer, potentially contributing to the increasing number of cancer cases worldwide.

Conclusion

Al, As, Cd, Cr, Mn, Ni, Pb, and Zn concentration in free-range chicken giblets was measured as a possible health risk for adults and children. As, Cr, Ni, and Pb concentrations exceeded the MAC limit in the giblets, while Cd was below the detection limit. The EDIs of the metals except Al, Mn, and Zn exceeded the MTDI limit. The cumulative risks of studied metals through consumption of the gizzard, kidney, and liver of free-range chicken did not exceed unity (THQs < 1), indicating that people will not experience significant risks in their consumption. Similarly, the calculated HI values were lower than 1. However, the CR of As, Cr, Ni and Pb exceeded the threshold limit, indicating a potential risk of cancer through their consumption. Given that free-range chickens are not selective in picking their food in the environment, a strict regime of proper disposal of waste products containing metals into the environment should be advocated and followed. It will assist in decreasing the amount of metals consumed by free-range chickens. The availability of mature local chickens caused delays in the research process. Gathering accurate data on the chickens' behaviour, health, or output was challenging due to a lack of data and fluctuating prices. The price of chicken also limited the number of samples analysed. Future studies should include other birds and some domestic animals.

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Data availability

Data will be made available on request.

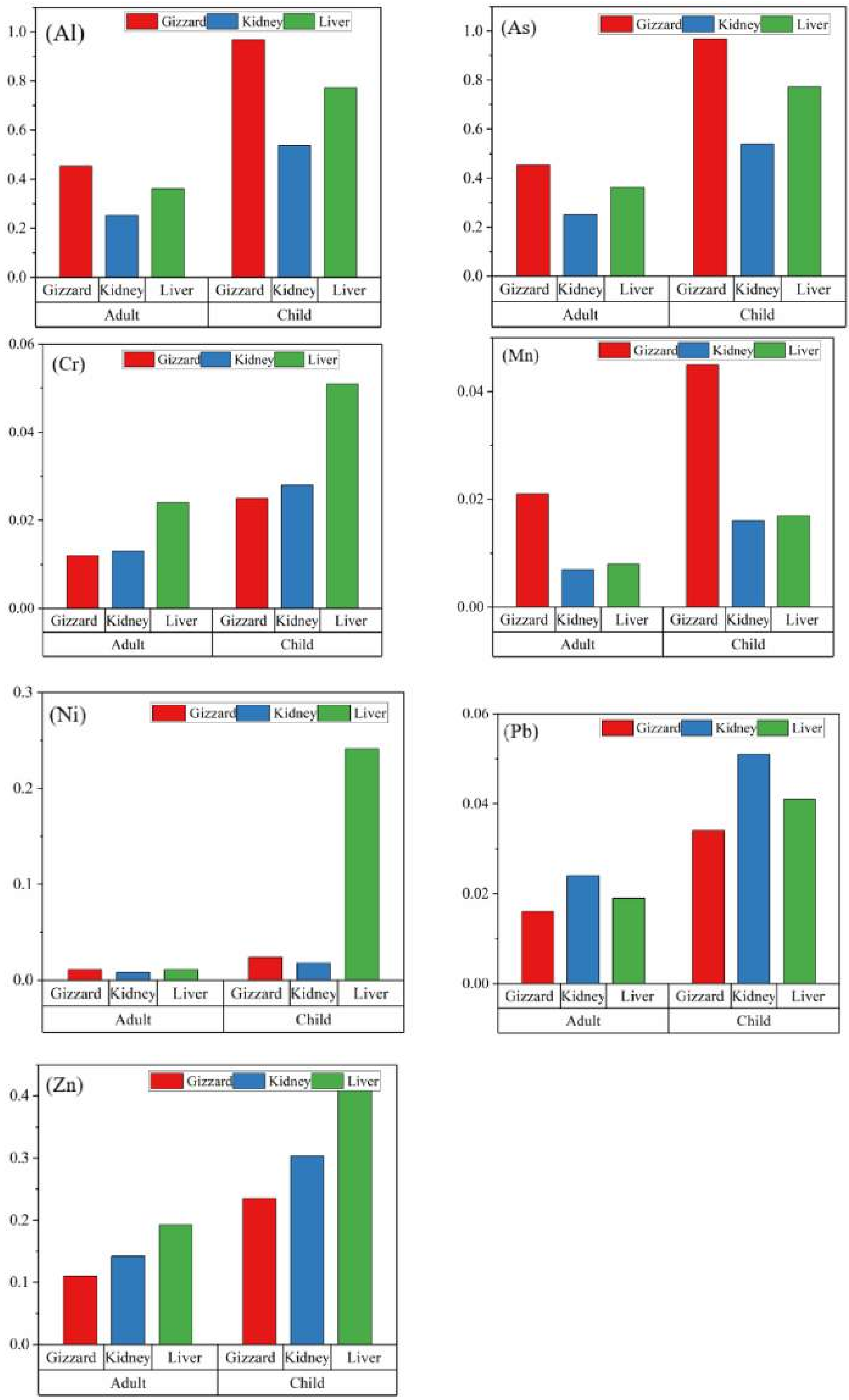

Fig. 5. Estimated Daily Intake (EDI) of Al, As, Cr, Mn, Ni, Pb, and Zn (mg/kg/day) in giblets.

Table 4
THQ for adults and children through the consumption of in the giblets of free-range chicken in the study areas.

	Giblets	Al	As	Cr	Mn	Ni	Pb	Zn	HI
Adult	Gizzard	4.54E-04	2.90E-02	3.89E-03	1.50E-04	5.71E-04	4.57E-02	3.66E-04	8.01E-02
	Kidney	2.51E-04	–	4.43E-03	5.22E-05	4.16E-04	6.79E-02	4.72E-04	7.35E-02
____	Liver	3.61E-04	3.35E-02	7.92E-03	5.55E-05	5.67E-04	5.48E-02	6.40E-04	9.78E-02
Child	Gizzard	9.69E-04	6.20E-02	8.32E-03	3.22E-04	1.22E-03	9.79E-02	7.83E-04	1.72E-01
	Kidney	5.38E-04	–	9.49E-03	1.12E-04	8.91E-04	1.45E-01	1.01E-03	1.57E-01
____	Liver	7.73E-04	7.18E-02	1.79E-02	1.19E-02	1.21E-03	1.17E-01	1.37E-03	2.22E-01

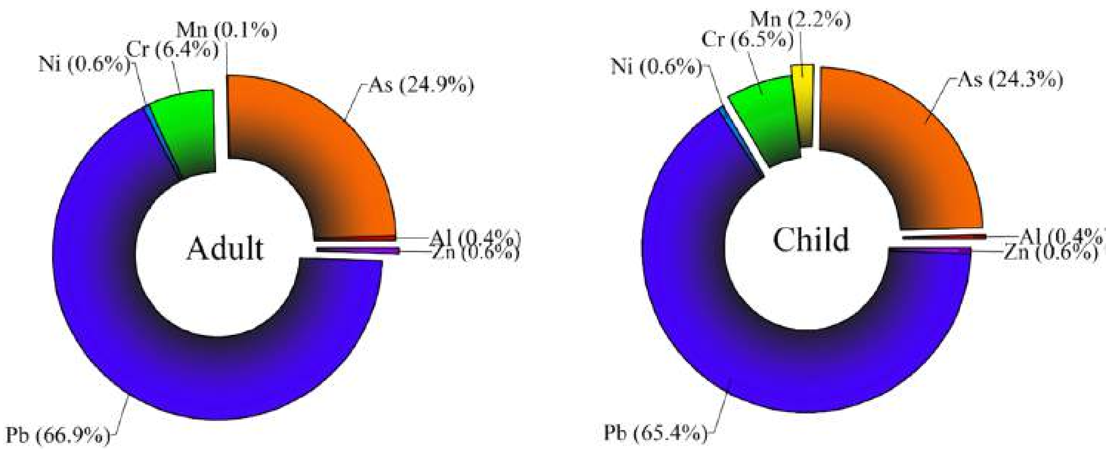

Fig. 6. Contribution of health risks caused by the different metals according to HI.

Table 5
Carcinogenic risk (CR) of heavy metals in the giblets of free-range chicken in Lokoja.

	Giblets	As	Cr	Ni	Pb	∑MCR
Adult	Gizzard	1.35E-02	6.00E-03	1.87E-02	1.36E-04	3.83E-02
	Kidney	–	6.50E-03	1.36E-02	2.04E-04	2.03E-02
____	Liver	1.50E-02	1.20E-02	1.87E-02	1.62E-04	4.59E-02
Child	Gizzard	2.85E-02	1.25E-02	4.08E-02	2.89E-04	8.21E-02
	Kidney	–	1.40E-02	3.06E-02	4.34E-04	4.50E-02
____	Liver	3.30E-02	2.55E-02	4.10E-01	3.49E-04	4.69E-01

Ethical approval and consent to participate

The research did not involve life animals, humans, in vitro, and other related issues where consent is needed.

CRediT authorship contribution statement

Jude Ehwevwerhere Emurotu: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Conceptualization. **Tenimu Adogah Abubakar:** Writing – review & editing, Software. **Loveth Chukwu:** Investigation, Formal analysis, Data curation. **Queen Ese Umudi:** Validation. **Victory Imokan Imumorin:** Investigation, Formal analysis, Data curation. **Gloria D. Paul:** Investigation, Formal analysis, Data curation. **Grace Unekwuojo Oboni:** Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtemin.2024.100209.

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