Accumulation Patterns and Risk Assessment of Metals and Metalloid in Muscle and Offal of free-Range Chickens, Cattle and Goat in Benin City, Nigeria

Emmanuel Temiotan Ogbomida[1], Shouta M.M. Nakayama[2], Nesta Bortey-Sam[2], Balazs Oroszlany[2], Isioma Tongo[3], Alex Ajeh Enuneku[3], Ogbeide Ozekeke[3], Martins Oshioriamhe Ainerua[3], Iriagbonse Priscillia Fasipe[1], Lawrence Ikechukwu Ezemonye[3], Hazuki Mizukawa[2], Yoshinori Ikenaka [2,4], *Mayumi Ishizuka[2]

[1]Ecotoxicology and Environmental Forensic Unit, National Centre for Energy and Environment, Energy Commission of Nigeria, University of Benin, P.M.B 1154, Benin City, Nigeria

[2]Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita ku, Sapporo 0600818, Japan

[3]Department of Animal and Environmental Biology (AEB), University of Benin, P.M.B 1154, Benin City Nigeria

[4]Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

* Corresponding author: Mayumi Ishizuka, e–mail: ishizum@vetmed.hokudai.ac.jp

Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18, W9, Kita–ku, Sapporo 060–0818, Japan

Tel: +81–11–706–6949; Fax: +81–11–706–5105

Abstract

The use of free range animals for monitoring environmental health offers opportunities to detect exposure and assess the toxicological effects of pollutants in terrestrial ecosystems. Potential human health risk of dietary intake of metals and metalloid via consumption of offal and muscle of free range chicken, cattle and goats by the urban population in Benin City was evaluated. Muscle, gizzard, liver and kidney samples were analyzed for Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb concentrations using inductively coupled plasma mass spectrometer (ICP-MS) while Hg was determined using Hg analyzer. Mean concentrations of metals (mg/kg ww) varied significantly depending upon the tissues and animal species. Human health risk estimations for children and adults showed estimated daily intake (EDI) values of tissues below oral reference dose (RfD) threshold for non essential metals Cd, As, Pb and Hg thus strongly indicating no possible health risk via consumption of animal based food. Calculated Hazard quotient (THQ) was less than 1 (<1) for all the metals analysed for both adult and children. However, Cd and As had the highest value of THQ suggestive of possible health risk associated with continuous consumption of Cd and As contaminated animal based foods. Hazard Index (HI) for additive effect of metals was higher in chicken liver and gizzard for children and chicken liver for adults. Thus, HI indicated that chicken liver and gizzard may contribute significantly to adult and children dietary exposure to heavy metals. Principal component analysis (PCA) showed a clear species difference in metal accumulation between

chickens and the ruminants. This study provides baseline data for future studies and also valuable evidence of anthropogenic impacts necessary to initiate national and international policies for control of heavy metal and metalloid content in food items.

Keywords: Heavy metals, Offal, Muscles, Hazard Quotient, Hazard Index

1.0 Introduction

Heavy metals and metalloids are pollutants that pose a great potential threat to the environment and human health on a global scale. They are intrinsic, natural constituents of the environment (Simone *et al*., 2012) and humans may promote their pollution through anthropogenic activities (Gall *et al*., 2015) such as extensive application of fertilizers or sewage sludge and pesticides in agriculture, waste disposal, atmospheric deposition, burning of fossil fuels, smelting, mining operations, electroplating, and discharge of wastewater from manufacturing industries including textile industry (Gall *et al*., 2015).

Heavy metals are widespread and highly persistent in the ecosystems due to their stability and non-degradable properties (Ali *et al*., 2013). They can enter the food chain via anthropogenic or natural contaminations of air, water or soil and accumulate in animals at the top of the food chain through bio-magnification effects to pose chronic toxicity and serious health risk to man. Heavy metal toxicity in man can diminish mental and central nervous system function, elicit damage to blood composition, as well as the kidneys, lungs, and liver, and reduces energy levels (Amirah *et al*., 2013). Long-term exposure may result in slow progressive physical, muscular and neurological degeneration that mimics Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis (Amirah *et al*., 2013).

In recent years, human exposures to heavy metals in Africa have risen as a result of an exponential increase in industrialization, urbanization, municipal solid waste generation and agricultural processes. Also uncontrolled illegal mining activities have added to the enormous amount of heavy metal contamination. The impact of these heavy metals on the environment can be a serious threats to the stability of the ecosystem and human health especially the African children as vulnerable group. In Sub-Sahara Africa environmental pollution by heavy metals has become a serious problem due to non-compliance and enforcement of existing environmental laws and regulations, inadequate monitoring capabilities, weak institutional structures and poor legal framework. In March 2010 large scale of lead poisoning due to artisanal/illegal gold mining activities occurred in Anka and Bukkuyum local government area of Zamfara state North-Western Nigeria leading to 163 deaths of which 111 were children under 5 years of age (MSF, 2010). Similarly, in May 2015, 28 children also died in Angwan Maijero and Angwan Karo, Madaka district of Rafi local government area of Niger State, Nigeria as a result of lead poisoning (MMSD, 2015).

Contamination of the environment and dietary intake of contaminated meat and meat products from food chain has been the most common and principal pathways of human exposure to heavy metals. Animals at the top of food chain may generally accumulate a large amount of heavy metals in their tissue, according to their age, size and feeding habits (Mahmood *et al*., 2012). In Nigeria, traditional rural animal production systems are mainly based on free-range (FMAWR, 2008). These animals could potentially pick heavy metals through grazing pesticide treated vegetation land, scavenging in open waste dumps for fodder, drinking polluted water from drains and streams and exposure to atmospheric depositions especially from automobile fumes and open burning of solid waste. These heavy metals can accumulate in organs and other fatty tissues, thus providing a major route for human exposure upon consumption. A recent study conducted by Bortey-Sam et al. (2015) evaluated human health risks from metals and metalloid via consumption of food animals in Tarkwa municipality in the western region of Ghana, an area predominantly known for artisanal gold mining activities. They indicated accumulation and distribution of heavy metals and metalloid in offal and muscles of chicken, goat and sheep with sufficient emphasis on species sensitivity and public health risk through consumption of these animal based foods. The study shows evidence of metal transfer from artisanal gold mining sites to free range animals thus, leading to accumulation of different metals in offal and muscles (Cd, Cr, Cu, Ni, Pb and Zn). They observed that metal accumulation was more pronounced in the liver and kidney of chicken than in muscles. This signifies sensitivity of chicken to heavy metals accumulation and the unique role of animal based food play in metals and metalloid transfer to man with possible health risks. While Bortey-Sam *et al.* (2015) succinctly compare health risks of metals and metalloid via intake of offal and muscles of free range animals from Tarkwa artisanal gold mining sites, recent studies in Nigeria focused mainly on human health risk assessment of some selected heavy metals via drinking water (Maigari et al., 2016), consumption of fishes (Orosun et al., 2016), cow meat (Ihedioha and Okoye, 2013), and illegal gold mining site (Olujimi et al., 2015). However, potential health impacts of municipal solid waste (MSW) in urban areas with specific reference to free range animals reared in proximity to the dumpsites have not been fully evaluated. Causal linkages between exposure to waste and health outcomes for some particular types waste are well established, but the impacts of MSW on free range animals still remain unclear or not prioritized as public health issues. MSW is a growing major challenge to many rapidly urbanizing Africa countries. Furthermore, the full extent of the burden of ill health attributable to exposure of free range animals and the consumption of their muscles and offal has not also be elucidated.

The present study address health risks of metals and metalloid of offal and muscles of free range animals from municipal solid waste sites in Benin City Nigeria, a major environmental problem of urbanisation, population growth and economic development. Free range animals are important in heavy metal studies as bio-indicators of the general environmental pollution status (Roggeman *et al*., 2013). Therefore, comprehensive study of heavy metal levels in tissues and offal of free range animals are needed to documented the safety of meat and meat products in Nigeria. Although heavy metal contamination have been documented in literatures, information concerning human health risk via consumption of meat and meat products contaminated with heavy metal in Benin City, Edo State still remain very scarce. Therefore, this study describes and compares accumulation, distribution and species sensitivity to heavy metals under different land use pattern. The accumulation of metals and metalloid in free range animals from different locations and ecological regions present a clear picture of environmental pollution status. The distribution pattern of metals in different organs using principal component analysis (PCA) showed species sensitivity to metal and possible human exposure through dietary intake. The outcome of this study will aid in the development of sustainable environmental management options of municipal solid wastes and health intervention policies for affected areas and many other developing countries.

2.0 MATERIALS AND METHODS

2.1 Study area

The study was conducted in Benin City, Edo State around markets, abattoirs and dumpsite areas (Fig 1). Benin City is located 6.3176°N, 5.6145°E and is the capital and largest City of

Edo State in Southern Nigeria. The city has a total area of 1,225 km^2 with an estimated human population of 1,147,188 (NPC, 2006). The city is a commercial centre strategically positioned as the gateway to the northern, eastern and western states of Nigeria. The notable economic activities include breweries, wood carving, traditional brass and bronze casting, wood and timber processing, printing and publishing. Major markets located within the city are Oba, New Benin, Oliha, Uselu, Agbado and Edaiken markets. Domestic and industrial wastes are often generated and discharged into the environment causing public health nuisances. Heaps of solid wastes are often seen littering the streets and market places. The disposal sites are capable of releasing large amounts of harmful chemicals such as heavy metals into the soil which easily found their ways into the food chain.

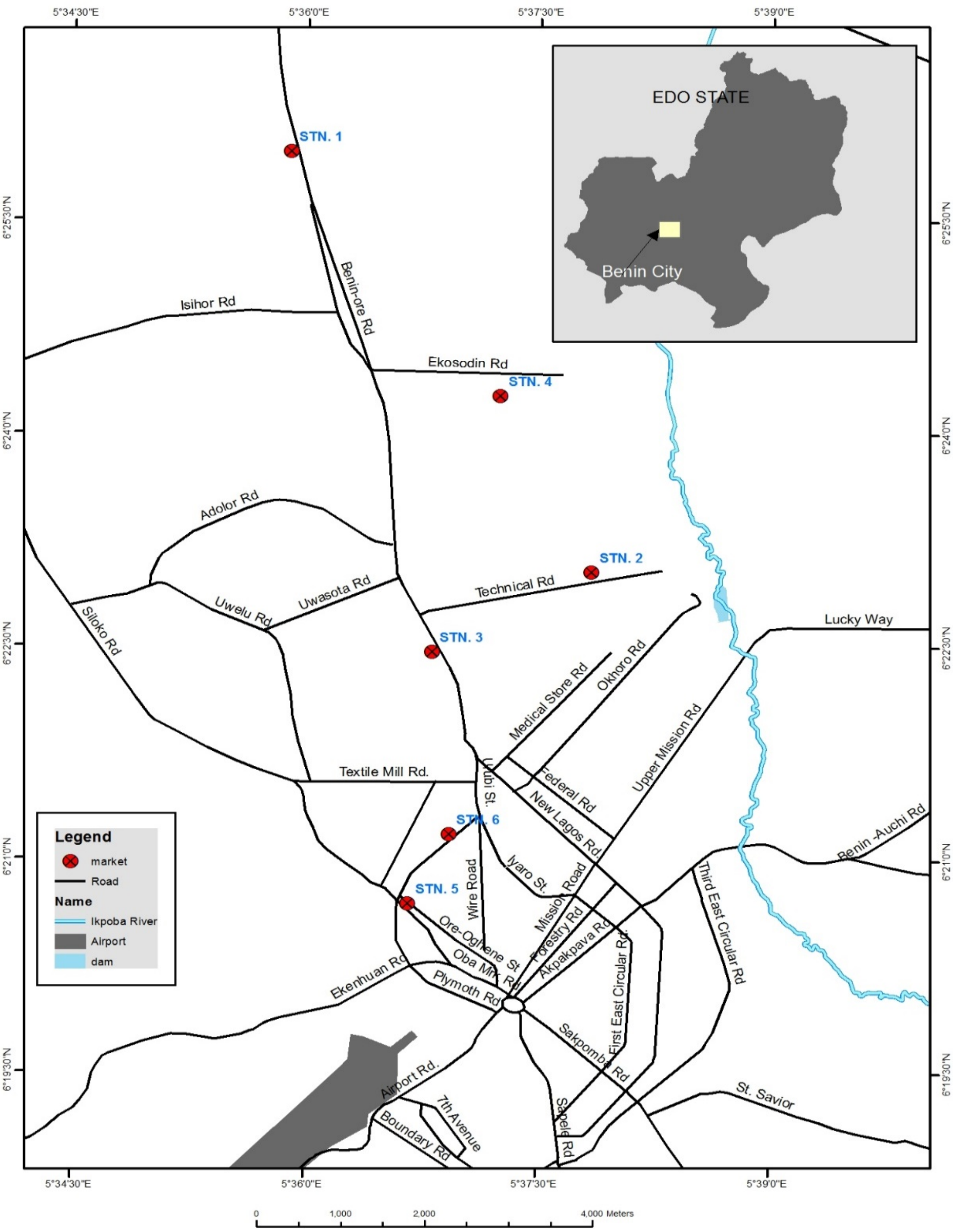

Fig 1: Map of Benin City showing sampling locations

2.2 Sample Collection

This study was carried out between August 2013 to March 2014 in Benin City, Edo State of Nigeria. Samples were collected from six locations in the three Local Government Areas which includes Ovia North-East Local Government Area (Oluku abattoir), Egor Local Government Area (Benin Technical College Road abattoir, Uselu market, University of Benin residential quarters, Ekiuwa market) and Oredo Local Government Area (Oliha market) of Edo State (Fig 1). Fresh samples of kidney, liver and muscle of free-range goats (*Capra hircus*) and cattle (*Bos Taurus*) were collected from the abattoirs while kidney, liver and muscle of free-range chickens (*Gallus gallus domesticus*) were collected from live adult chickens after exsanguination and dissection in the laboratory. Samples were kept frozen in labelled plastic bags in the National Centre for Energy and Environment laboratory, University of Benin and were later tansported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan and stored in -20ºC until analysis. All procedures used in this experiment were performed according to the guidelines of the Committee of Animal Care and Use, University of Benin.

2.3 Sample Preparation and Metal Extraction

All materials and instruments used in metal extraction were washed in 2 % nitric acid (HNO_3) and rinsed at least twice with distilled water. Metals were extracted from liver, kidney and muscle by acid digestion using a closed microwave digestion system (Speed wave MWS-2; Berghof, Germany) according to the method of Nakayama *et al.* (2011). Briefly, 0.5 g of thawed liver, kidney and muscle samples each were placed in a prewashed DAP-60K digestion vessel. 5 ml of 65 % HNO_3 (Kanto Chemical Corp., Tokyo, Japan) and 1ml of (30 %) hydrogen peroxide, H_2O_2 (Kanto Chemical Corp., Tokyo, Japan) spectrometry grade were added. The digestion vessels were capped and placed into a 10-position turntable conditions followed by a ramped temperature program: ramp to 160°C (5 min hold); and increase to 190°C (15 min hold). After digestion in the microwave for 52 min, the samples were cooled and transferred into plastic tubes and diluted to final volume of 10 ml with distilled deionized water (Milli-Q water). A reagent blank was also prepared using the same procedure.

2.4 Metal Analysis

The quantification of metals in the digested samples were determined using an Inductive Coupled Plasma-Mass Spectrometer (ICP-MS) model 7700 series, Agilent technologies, Tokyo, Japan). The instrument was calibrated using standard solutions of respective metals to establish standard curves before metal analysis. All chemicals and standard stock solutions were analytical reagent–grade (Wako Pure Chemicals, Osaka, Japan), while the water was bi distilled and deionized (Milli-Q; Merck Millipore). The detection limits (µg/L) of chromium (Cr), manganese (Mn), Iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb) were 0.0024, 0.0118, 0.0187, 0.0008, 0.0081, 0.0042, 0.0161, 0.0022, 0.0005 and 0.0022 respectively. Concentrations of metals were expressed in mg/kg wet weight (mg/kg ww).

2.4.1 Total Mercury Analysis

The concentration of total mercury (Hg) was measured by thermal decomposition, gold amalgamation and atomic absorption spectrophotometry (Mercury Analyzer, MA-3000; Nippon Instruments Corporation, Tokyo, Japan), after preparation of calibration standards. Recovery rates of Hg for the certified reference material, DOLT-4 (Dogfish liver, the National Research Council, Canada) ranged from 92 % to 103 % (94.3 ± 4.2 %). The detection limit of total mercury (Hg) was 2.0 pg.

2.5 Quality Assurance and Quality Control

All analytes were subjected to stringent quality control methods. Before sample analysis, appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. Double distilled deionized water was used throughout the study. Glassware were properly cleaned, and reagents were of analytical grade. Reagents blank determinations were used to correct the instrument readings. Replicate blanks and standard reference materials (SRM), DORM-3 (Fish protein, the National Research Council, Canada) and DOLT-4 (Dogfish liver, the National Research Council, Canada) were used for method validation and quality control. Replicate analysis of these reference materials showed good accuracy (relative standard deviation, RSD, $\leq$ 3 %) and recovery rates ranged from 80 % to 115 %.

2.6. Human health risk assessment

Risk assessment was done based on the mean concentrations of carcinogenic and non-carcinogenic metals determined in the meat samples using United States Environment Protection Agency (USEPA, 2015) human health risk assessment models. It was assumed that the ingested dose is equal to the adsorbed contaminant dose and that cooking has no effect on the toxicity of heavy metal (Copat *et al*., 2013). Health risk estimates for heavy metals concentrations in cattle, goat and chicken tissues were computed using three basic standard indices: Estimated Daily Intake (EDI), Hazard Quotient (THQ) and Hazards index (HI). Two hypothetical age/weight categories were used. 30 kg was assumed for children and 60 kg for adults (Ihedioha and Okoye, 2013).

2.6 Data Analysis

2.6.1 Estimated daily intake (EDI) of toxic metals

The estimated daily intakes (EDI) of heavy metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg and Pb) was expressed as mg/kg/day and was dependent on both the concentration of heavy metals in offal or muscle and the amount of consumption. The EDI of metals for children and adults were determined by the following equation according to Bortey-Sam *et al*. (2015).

$$EDI = \frac{MC \times FDC}{BW} \quad \text{Eqn. 1}$$

where MC is average concentration of metal in food (µg/g, on fresh weight basis); FDC represents the average offal/muscle daily consumption in this region (g/person/d); BW is the average body weight. It was assumed that local inhabitants consumed an average liver, muscle and gizzard of 150 and 100 g/day for adult (60kg in BW) and children (30 kg in BW) respectively (Bortey-Sam *et al*., 2015). For kidney, it was assumed that inhabitants consumed 10 and 8 g/day for adults and children respectively (Ihedioha and Okoye, 2013), because the size of kidneys are relatively smaller and is not the favorite compared with liver, muscle, or gizzard. The metal intakes were compared with the tolerable daily intakes for metals recommended by FAO/WHO (2010).

2.6.2 Target Hazard Quotient (THQ)

The Target Hazard Quotient (THQ) was used to assess risk associated with non-carcinogenic and carcinogenic health effect. The health risks from consumption of offal and muscle of chicken, goat and cattle by the local inhabitants were assessed based on the hazard quotient

(THQ). The THQ is a ratio of determined dose of a pollutant to a reference dose level. If the ratio is less than 1, the exposed population is unlikely to experience obvious adverse effects. The method of estimating risk using THQ was provided in the USEPA Region III risk-based concentration table (USEPA, 2007), and is based on the equation below:

$$THQ = \frac{EFr \times ED \times FIR \times MC}{RfD \times BW \times AT} \times 10^{-3} \quad \text{.......... Eqn. 2}$$

where THQ is target hazard quotient; EFr is exposure frequency (365 days/year); ED is exposure duration (70 years); FIR is food ingestion rate (g/person/d); MC is average concentration of metal in food (μg/g, on fresh weight basis); RfD is the oral reference dose (mg/kg/d); BW is the average body weight, adult (60 kg); children (30 kg); AT is the average exposure time (365 days/year number of exposure years, assuming 70 years in this study). Oral reference doses (RfD) were based on 3E-04, 1E-03, 5E-04, 4E-03, 3E-01, 4E-02, 1.4E-01, 2E-02, 7E-01, 15E-1 mg/kg/d for As, Cd, Hg, Pb, Zn, Cu, Mn, Ni, Fe, Cr respectively (USEPA IRIS, 2007). There is no consensus about the RfD for Co, However, the RfD for Co was estimated as 4.3E-02 (Food and Nutrition Board, 2004).

2.6.3. Hazardous Index (HI)

To estimate the human health risk for more than one heavy metal, the hazard index was calculated by the summation of the hazard quotients for all heavy metals in the Equation below.

$HI = \Sigma THQ_n$ …………….Eqn 3

It assumes that the magnitude of the adverse effect will be proportional to the sum of multiple metal exposures. It also assumes similar working mechanisms that linearly affect the target organ. When HI is equal to or less than one ($HI \leq 1$), it indicates no appreciable health risk, while if $HI > 1$, then it indicates a reason for health concern (Cao *et al*., 2015). Hence, $HI < 1$ means no hazard; $1 > HI < 10$ means moderate hazard while greater than 10 means high hazard or risk (Ukoha *et al*., 2014).

2.6.4 Statistical analysis

Statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Illinois, USA) after data were normalized by log transformation. To analyze differences in the distribution of metals in tissues of free-range chickens, cattle and goats, analysis of variance (ANOVA) and Tukey was done and differences were considered statistically significant with p value < 0.05. Principal component analysis (PCA) based on log transformed data was done to determine the distribution pattern of metals in organs, using JMP statistical software v.10 (SAS Institute).The principal components were extracted with eigen values > 1. Statistical differences between the different organs (liver, muscle, gizzard and kidney) were determined by one-way analysis of variance (ANOVA). The limit of significant level was accepted at $p < 0.05$.

3.0. Results and Discussion

3.1 Concentration of Metals and Metalloid in Offal and Muscle

The mean concentrations of these metals in offal and muscle of free-range chickens, cattle and goats from Benin city are presented in Table1. The composition profile of the metals in the three animal food species are Cr (0.050 - 0.29), Mn (0.13 - 3.6), Fe (10 - 310), Co (0.0040

- 0.054), Ni (0.013 - 0.049), Cu (0.50 - 88), Zn (4.4 - 47), As (0.0040 - 0.081), Cd (0.0010 - 0.89), Pb (0.0090 - 0.59) and Hg (nd - 0.019) mg/kg ww. The results showed that chicken liver accumulated the highest levels of Fe, Mn and Hg, chicken kidney (Pb, Cd and As) and chicken gizzard (Ni and Cr) while cattle liver accumulated Co, Cu and Zn. This findings comform with Demirezen and Uruc (2006) who reported that the primary site of heavy metals are the liver and kidney due to exposure and physiological responses of animals to detoxify the system. The reason for high accumulation of metals in the gizzard could be attributed to the function of the gizzard which is the primary reservoir of food materials and organ for grinding up food partilces. Free range local chickens according to Nesheim *et al.* (1979) have been found to harbour the greatest pollutants in their body system as they have no oral discrimination for food hence pick whatever they find edible in the environment. In this study free-range chicken accumulated more metals than the cattle and goat which can be attributed to their foraging dietary habits. The high levels of Pb, Cd, Hg, As, Ni, Cr, Mn and Fe in the offal of the chickens could have come from anthropogenic activities in the environment and feeds fed on by the chickens. Several studies have shown that diet is the primary pathway for metal accumulation in animals and man. For example, toxicological investigations on bovines, ducks and poultry have shown a direct correlation between metal concentrations in animal feed and animal tissues accumulation (Sedki et al., 2003; Kim and Koo, 2007). Also, it has been reported that rice and vegetables grown in mining areas contaminated by heavy metals have posed a great potential health risk for consumers, who suffered from serious cancers (Zhuang et al., 2009). Plants uptake from soil contribute to the circulation of heavy metal in the food chain through their active and passive absorption, accumulation in tissues as well as subsequent grazing by animals that depends on them for nutrients or consumption by man. This reflect that metal-rich diets lead to high tissue metal accumulation. Metal accumulation in liver and kidney also reflect short-term exposure and are most appropriate target organ to test metals.

3.1.1 Human Essential Metals

The 11 elements of highest concern within the European Community are: As, Cd, Co, Cr, Cu, Hg, Mn, Fe, Zn, Ni, and Pb (Stankovic and Stankovic, 2013). Some of these elements are actually necessary for humans in trace amounts (Co, Cu, Cr, Mn, Ni), while more than necessary amounts of these trace elements and some others are carcinogenic or toxic, affecting among others, the central nervous system (Hg, Pb, As, Mn), the kidneys or liver (Hg, Pb, Cd, Cu) or skin, bones, or teeth (Ni, Cd, Cu, Cr) (Rai and Pal, 2002; Chen et al., 2008; Lavery et al., 2009; Jovic et al., 2012; Stankovic and Jovic 2012; Markovic et al., 2012). Essential metals are significant to humans because they are important in various metabolic enzymes and constituents of cells (Maret, 2016). The frequent consumption of animal based food contaminated with heavy metals could increase the level in humans. Large doses of essential metals can damage living organisms or in some cases results in toxic effects (Nagajyoti *et al*., 2010).

Cu is a component of various enzymes (Amaral et al., 2008; Osredkar and Sustar, 2011). It is involved in collagen synthesis and in the normal development of connective tissues, nerves and immune system (Amaral et al., 2008). High intake has been recognized to cause adverse health problems such as liver and kidney damage (Amaral *et al*., 2008). Concentration of Cu was highest in cattle and chicken liver (Table 1). This is because the liver act as the primary storage organ for Cu and maintains Cu homeostasis for animal. In the present study the results shows that the average concentration of Cu in cattle liver (88.016 ± 36.349 mg/kg ww) is higher than the permissible limits of 20 mg/kg set by USDA (2006) and ECR (2006) and also 30 mg/day maximum limit set by WHO (1996).

Mn is an essential element for both animals and plants; its deficiency results in severe skeletal and reproductive abnormalities in mammals (Sivaperumal *et al*., 2007). Mn concentration was found to be high in livers and kidneys of chicken and cattle liver. The mean concentrations of Mn ranged from 0.130 ± 0.037 (chicken muscle) to 3.607 ± 1.198 mg/kg ww (chicken liver). Mn levels in chicken liver, kidney and cattle liver and were above the WHO (1996) reference standard of 0.5 mg/kg. Mn has been found to be toxic in excess; in brain it can cause a Parkinson-type syndrome (Aschner, 2000).

Fe is one of the most abundant transition element, and probably the most well known metal in biologic systems participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. High levels of iron may lead to tissue damage, as a result of the formation of free radicals. The concentration of Fe in this study was highest in chicken liver and kidney. The mean concentration of Fe ranged from 10.349 mg/kg ww in cattle muscle to 305.484 mg/kg ww in chicken liver (wet weight). This result reflect the dominance of Fe concentration observed in other animal based foods by Medeiro *et al*. (2012). The most serious forms of Fe overload is acute poisoning while chronic Fe intoxication is associated with genetic and metabolic diseases leading to repeated blood transfusions (Fraga and Oteiza, 2002).

Zn is the most abundant intracellular component which is needed to maintain some important biological functions such as genetic stability, gene expression, DNA repair and programmed cell death (Hussein and Khaled, 2014). High levels of zinc can cause pancreatitis, anemia, muscle pain, and acute renal failure (Pais and Benton Jones, 1997). In this study the mean concentration of Zn ranged from 4.430 mg/kg ww in chicken muscle to

46.704 mg/kg ww in cattle liver. This result conform with Okoye and Ugwu (2010) who reported elevated zinc levels in goats bred in Nigeria. The high concentrations of Zn in animal based food can be attributed to food chain transfer. The values of zinc reported in this study were below the Codex Alimentarium Commission maximum permissible limit of 50 mg/kg for muscle and 80 mg/kg for edible offals (Codex, 1995).

Cr is an important element known to enhance the action of insulin in man (Mertz, 1998), a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body (Porte *et al*., 2003). The mean concentrations of Cr in this study ranged from 0.051 (chicken kidney) to 0.286 mg/kg ww (chicken gizzard). Our data is similar to Iwegbue, *et al*. (2008) who reported 0.33 ± 0.14 mg/kg in chicken meat consumed in southern Nigeria. Cr detected in the gizzard of chicken could be attributed to chicken diet.

Ni plays an important role in the biology of plants and animals. Overexposure to Ni can cause decreased body weight, heart and liver damage and skin irritation (Homady *et al*., 2002). The average concentration of Ni observed in this study ranged from (0.017 ± 0.011-0.049 ± 0.037 mg/kg ww) in chicken (0.019 ± 0.003 - 0.030 ± 0.020 mg/kg ww) in cattle and (0.013 ± 0.005 - 0.028 ± 0.020 mg/kg ww) in goat. The levels of Ni in the kidneys of chicken, cattle and goat showed no significant difference ($P > 0.05$) while the livers and muscles of cattle showed significant difference when compared with chicken and goat. Chicken gizzard and muscle had the highest value of Ni. The observed levels in this study were below the 0.5 mg/kg maximum permissible limit of Ni in food according to FAO/WHO (2000).

3.1.2 Human Non-Essential Metals

Human non essential metals Cd, As, Pb and Hg have no biological role in living organisms but are tolerated at very low concentration and also inhibit metabolic activity at higher concentrations (Pandey and Madhuri, 2014) . They are potentially toxic to living organisms. Animals are contantly exposed to these toxicant through dietary intake. As shown in Table 1 the concentration of Cd ranged from 0.890 ± 1.035 mg/kg ww in chicken kidney to 0.001 ± 0.00 mg/kg ww in muscle of cattle and goat.

The range of Cd level in this study is lower than result obtained by Ihedioha and Okoye (2013) in swine and broiler chicken (ND - 40.14 mg/kg ww). The levels of Cd observed in free range chicken kidney were below the maximum permissible limit of 1.0 mg/kg (FAO/WHO, 2000). However, Doganoc (1996) observed higher levels of Cd in the liver and kidney of chicken which exceeded the official tolerance levels. The ability of liver and kidney to accumulate high concentration of Cd is a common feature of chickens and many other animals and may be due to the detoxification function of the organs (Stoyke *et al.*, 1995). Similar levels of Cd has also been reported by Okoye and Ugwu (2010) in liver (0.35 mg/kg) and kidney (0.83mg/kg) of goats from Nigeria. Cd is presently listed as number 7 of 275 of the most hazardous substances in the environment, behind As, Pb and Hg (ATSDR, 2013). Chronic Cd poisoning impair calcium metabolism leading to softening of bones, fractures, and skeletal deformations (Stankovic *et al.*, 2011). Liver and kidney tissues are the two main sites of Cd storage and these organs accumulate considerable amounts of Cd, about 40 - 80 % of the body burden (WHO, 2007).

Pb is the second element (after arsenic) on the ASTDR's top 20 list of the most poisoning heavy metals (ASTDR, 2013). Its target organs are bones, brain, blood, kidneys, reproductive and cardiovascular systems, and thyroid gland (Homady *et al.*, 2002). The levels of Pb in the offal and muscle of free range cattle, goat and chicken ranged from 0.58826 mg/kg ww in chicken kidney to 0.009 mg/kg ww in goat muscle (Table 1). Pb is the most frequently reported causes of accidental poisoning in domestic animals, with cattle as the most commonly affected species because they are very inquisitive and commonly ‘taste test’ new finds (Khalafalla *et al*., 2015). Absorbed Pb is stored mainly in the liver and kidney, and like Cd, it accumulates in tissues of animals (Khalafalla *et al*., 2015). However, there was significant differences when the concentration of Pb in chicken kidney and liver were compared with goat kidney and liver (Tukey test: $p < 0.05$) Table 1. Similarly, the level of Pb in the muscles were statistically different (Tukey test: $p < 0.05$). Apart from the chicken kidney, the gizzard was second highest in the accumulation of Pb. This could be attributed to the feeding pattern of chicken. In this study, concentration of Pb in the kidney (0.588 ± 0.703 mg/kg ww), and gizzard (0.239 ± 0.327 mg/kg ww) of free range chicken were slightly above the recommended limits of 0.5 and 0.1 mg/kg ww for offal and muscle by ECR (2006) and USDA (2006) respectively.

The result of this study indicated that the highest concentration of Hg was found in chicken liver (0.034 ± 0.036 mg/kg ww) and kidney (0.030 ± 0.031 mg/kg ww) while the lowest concentrations was found in cattle liver (0.001 ± 0.000) and goat muscle (0.0010 ± 0.002) mg/kg ww respectively. This is in conformity with Landis *et al.* (2010) that birds accumulate Hg in the kidney and liver with less in the muscle. The concentrations of Hg in the kidney of free-range chicken, cattle and goat were not statistically different (Tukey test; $p > 0.05$) However, levels of Hg in chicken liver were significantly different ($P < 0.05$) when compared with the levels in the livers of cattle and goat. In the muscle, the levels were significantly different ($p < 0.05$) with high value in chicken muscle. In this study the values of Hg in offal and muscles were below the permissible limit of 0.05 mg/kg established by ECR (2006) and USDA (2006).

The metalloid As was detected in all the tissues and offal samples of the three animal foods. Concentrations of As in muscle and offal of free-range chicken, goat and cattle ranged from (0.040 ± 0.034-0.081 ± 0.073 mg/kg ww), (0.007 ± 0.011-0.008 ± 0.011 mg/kg ww) and (0.007 ± 0.011 -0.008 ± 0.004 mg/kg ww) respectively (Table1). The distribution of As in liver, kidney, gizzard and muscle of chicken was found to be higher than that of goat and cattle ($p < 0.05$). Mean concentration of 0.08 mg/kg ww in the kidney of free-range chicken in Benin City was found to be higher than the USDA (2006), ECR (2006) and SAC/MOHC (2005) standard of 0.05 mg/kg.

3.1.3 Principal Component Analysis (PCA) of Metals Distribution

Principal component analysis (PCA) of heavy metals and metalloid distribution in offal and muscle samples of animal based food was performed and the results are presented in Fig. 2. PCA was used to determine the multivariate structure of the data, to highlight the possible trend and to further identify the potential source of heavy metals contamination. In the interpretation of PCA patterns, factor loadings greater than 0.71 are typically consider excellent while those less than 0.32 are regarded as very poor (Nowak, 1998). Factor loading determine the relationships between the variables and each factor. The first principal component (PC1) of metals and metalloid in liver of cattle (Fig. 2) showed 38.7 % of the total variance with the loading of the variables Mn, Fe, Ni, Zn and Pb. Fe and Zn in PC1 were highly associated, displaying high factor loading values (0.827 and 0.843) suggesting metals may come from the same source. Also, zinc and iron share common dietary sources.The second component (PC2) accounts for 14.7 % of the total variance of heavy metals in the chicken liver and dominated by Co, As and Cd while the third component PC3 accounts for 12.5 % of the total variance in goat liver with a positive loading for Cu. The first principal component which explains 38.7 % may be controlled by a long term anthropic activity such as municipal solid waste found to be seriously contaminated by toxic metals (Naveedullah *et al*., 2013) such as electronic waste in the area for a long period of time in agreement with the clustering of variables in Group I. Also metallic zinc and iron are used in a wide variety of applications but when exposed to the atmosphere may be subject to corrosion that may result in the slow release of small amounts of zinc and iron into the environment. The PCA results also showed a clear separation between chicken (F) grouped on one side and the ruminants; goat (G) and cattle (C) clustered on the other side. The results obtained from PCA of kidney of the three animal based foods Fig 2 indicates that Mn, Co, As, Cd and Pb are associated with high factor loadings of 0.857, 0.823 and 0.776 (Co, Cd and As) in cattle kidney. This association strongly suggests that these variables have a similar source and may be attributed to cattle grazing in polluted environment or drinking wastewater with heavy metals. The first PC which explains 33.5 % of the total variance and loads in the cattle kidney confirm the interpretation of their anthropogenic origin. The occurrence of Cd and Hg likely reflects the increased use of nickel-cadmium batteries in electronic items and a variety of Hg-containing devices and lamps such as compact florescence lamps (CFLs) which are disposed in municipal solid waste. The second component (PC2) showed 15.6 % total variance of heavy metals in chicken kidney which contributes Cu and Zn with factor loading of 0.840 and 0.784. Cu and Zn may be controlled by a long-term anthropic activity such as pesticides usage (Yang *et al*., 2014). Copper is usually considered as a marker element of environmental waste dumps, domestic waste water, combustion of fossil fuels and wastes, wood production, phosphate fertilizer production, and natural sources. The third component (PC3) alone explains 11.6 % of the variance of Cr and Ni in the goat kidney while Hg showed negative loadings. In the cattle muscle the first component (PC1) with 33 % of variance comprises positive factor loading of Mn, Fe, Cu and Zn which could be linked to anthropogenic and natural sources with some contributions from the municipal waste system. The second component (PC2) explains 19.3 % of the total variation, which exhibited a high positive factor loading of 0.755, 0.815 and 0.955 on Ni, Cd and Pb. These are significant environmental pollutants with anthropogenic origin especially from industry, production of lead-acid batteries, metal products (solder and pipes) and poor urban waste management which are common in developing countries. The third component (PC3) showed a high positive factor loading with 14 % variance of As and Hg. This suggest that levels of these toxic metals can be primarily attributed to anthropogenic influences. Animals are constantly exposed to As and Hg through contaminated drinking water, feedstuff, grasses, vegetables etc. However, arsenic has been one of the most common causes of inorganic chemical poisoning in farm animals and also a main threat to human health. Africa today is becoming a dumping ground for electronic waste especially used computers, mobile phones etc from developed countries which contains hazardous metals such as lead, cadmium and mercury. All these electronic wastes subsequently end up in municipal waste dump sites which consists of a mix of combustible and non-combustible materials and subsequently over time contribute to increased environmental problem.

3.2 Human Health Risk Assessment

3.2.1 Estimated Dietary Intake of Metals

The Estimated Daily Intakes (EDI) of metals and metalloid taken by an adult or children per day through the consumption of free range chicken, cattle and goat offal and tissue are presented in tables 2. Meat and edible offal are considered a significant part of most diet in Nigeria. They serve as major sources of animal derived protein, vitamins, essential trace elements and fatty acids. However, meat and edible offal consumption had been identified as an important pathway of human exposure to pollutants. The degree of heavy metal toxicity to human population depends upon their daily intake. The EDI values for human non essential metals Cd, As, Pb and Hg for adults person of 60 kg body weight show that chicken liver had the highest EDI value. The EDI values obtained were generally lower than the respective oral reference dose (RfD) threshold for human non essential metals Cd, As and Pb except in Hg. Our results strongly indicate no possible health risk associated with animal based food consumed by the local population of Benin city. The RfD threshold dose or intake is an estimate of a daily exposure to the human population that is likely to be without an appreciable adverse risk of deleterious noncancer effects when exposure is long-term (lifetime) intake. If the predicted intake is less than the oral reference dose (THQ < 0.1), it indicates almost no possibility of an adverse health effects. However if the intake exceeds the reference dose (THQ > 0.1) does not necessarily imply that adverse health effects are expected only that a conservative reference oral dose (RfDo) is exceeded. The results of this study are similar to those obtained in an earlier study on offal and muscles of sheep, goat and chicken (Bortey-Sam *et al*., 2015) where they reported low daily intakes of As, Cd, Hg, Pb and Mn in food animals compared to the provisional tolerable daily intake guidelines. Chronic exposure to low doses of Cd, As, Pb and Hg above their safe threshold in humans and animals could therefore result to many non- carcinogenic hazards such as neurologic involvement, headache and liver malfunctions. For human essential heavy metals Co, Cr, Cu, Mn, Fe, Zn and Ni, the estimated daily intake of Co, Cr, Cu, Mn, Fe, Zn and Ni in this study ranged from 0.005 - 0.135, 0.008 - 0.716, 0.698 - 220.041, 0.185 - 9.018, 13.808 - 229.838, 4.117 - 116.760 and 0.005 - 0.123 μg/kg bw/day respectively. Based on this values, the EDI is lower than the RfD guidelines which strongly showed that there is no serious health risk via consumption of these food based animals. The calculated EDIs of As, Cd, Hg, Pb and Mn in offal and muscle samples of the three food animals from Benin city were below the WHO (2000), FSA (2006) and FAO/WHO (2010) tolerable daily intakes (Table 2). However, the EDI values in children were higher than the adult values. Therefore, children are especially more vulnerable to acute, sub-acute and chronic effects of ingestion of chemical pollutants, since they (children) consume more (twice of the amount) of food per unit of body weight as adults (ENHIS, 2007). As a result, intakes of these toxic metals through food could be higher for children in Benin city. The EDI values from other studies agreed with our findings. Specifically, the findings of Tongo and Ezemonye (2015) on predicted EDI values for children associated with residual pesticide levels in edible tissues of slaughtered cattle in Benin City conform with our current findings. Also, Onwukeme et al. (2014) and Oforka et al. (2102) predicted the risk of heavy metals via consumption of cow meat and offal in Enugu and chicken meat in Port-Harcourt, Nigeria. However, our findings in this study regarding metals contents in meat and offal suggest that their consumption are not free of risks as they may present detrimental health concerns through a lifetime. Due to interspecific differences however, the EDI levels of heavy metals were higher in chicken than other species.

3.2.2 Target Hazard Quotient (THQ)

Estimated health risk from consumption of offal and muscle from chicken, cattle and goat were assessed based on the THQ. A THQ value greater than 1 would indicate that a potential health risk may exist. THQ value in this study was less than 1 which means the exposed population is unlikely to experience any obvious adverse effects (USEPA, 2000) from the intake of individual metals through the consumption of offal and muscle (Fig. 3). Generally, consumption of muscle tissue poses less health risk to man compared to kidney and liver because it accumulates low level of metal. However, muscle tissue seldom come in direct contact with metals as it is totally covered externally by the skin that in many ways prevent the penetration of metals. It is also not an active site for detoxification, and therefore, transport of metals from other tissues to muscle does not seem to arise (Elnabris *et al*., 2013). Our findings also indicates that the severity of metals and metalloids are more in liver and kidney. THQ for Cd and As were highest in chicken liver and gizzard but less than 1 in both adults and children. This may indicate potential significant health risk associated with continuous consumption of chicken liver and gizzard. Therefore, some attention should be paid for the Cd and As content in chicken because their THQ values for adults and children are not far below the threshold value of 1. The higher levels of the cadmium shown in the chicken liver relative to other tissues may be attributed to the high coordination of metallothionein protein with the cadmium. In addition, the liver act as the principal organ responsible for the detoxification, transportation, and storage of toxic substances. Also, feed habits and composition can influence the retention of heavy metals in the liver. Therefore continuous consumption of chicken liver may lead to biomagnification suggesting possible significant risk to man. The of the three animal based showed lowest values an indication that there might be no public health risk from the consumption of muscle of free-range chicken, cattle and goats. Our findings agrees with Tyokumbur (2016) and Ihedioha and Okoye (2013) who reported THQ value less than 1 for chicken and cow meat. The essential metals Cu, Zn, Fe and Mn were also clearly below all the permissible limits for human consumption.

3.2.3 Estimated Hazard Index (HI)

The hazard index (HI) of the mixture of different non-essential metals Cd, As, Pb and Hg was calculated by adding together all the component hazard quotients (THQ). The potential health effects of the mixture were further analyzed and investigated if the HI value is equal to or greater than 1. Hence, HI < 1 means no hazard; 1 > HI <10 means moderate hazard while greater than 10 means high hazard or risk (Ukoha *et al*., 2014). This approach assumes that all components have similar joint action for residents ingesting metals via consumption of tissues and offal of free range chicken, cattle and goat. The calculated HI values for both adults and children are given in Figure. 4. The HI for human non essential metals Cd, As, Pb and Hg revealed that chicken liver (2.19) and chicken gizzard (1.17) for children were greater than 1. This suggests that there is overtly adverse health effects from the consumption of chicken liver and gizzard. In adult, HI value showed that chicken liver (1.64) and gizzard (0.88) suggests higher health risk from the consumption of chicken liver than chicken gizzard. This implies that when metals combined they pose a greater health risk than any lone metal. All heavy metals analyzed had a hazard quotient of less than one (THQ < 1). The greater the value of (THQ) and (HI) (if > 1), the greater the level of risk associated with offal and muscle consumption. Our results agreed with MahMoud et al. (2014) that reported HI greater than 1 for Cd indicating high potential risk to the Egyptian consumers.

4.0 Conclusion

This study was developed to provide baseline information on heavy metals and metalloid concentration in free range animals; chicken, cattle and goats sampled in Benin City, Edo State, Nigeria. As expected, metal concentrations showed a great variability. The levels of metals in the offal and muscle suggested that significant differences existed in the concentrations of elements across offal and muscles of animal species. It was observed that free-range chickens reared in Benin City accumulated large quantities of Cd above maximum permissible limits compared to cattle and goats. This may be largely related to the organism mobility, food preferences, or other factors with respect to environment pollution most likely from dumpsites. The estimation of risk showed that adverse health effect may occur from prolonged consumption of chicken liver with high concentrations of Cd and may lead to accumulation in the human body thereby causing metal toxicity. Overall, our findings show that consumption of chicken liver and gizzard present more health than muscle tissue especially to children. Contamination is due mainly to environmental pollution as the animals are free ranging. Therefore, it is strongly recommended that appropriate steps should be taken to identify ways to reduce the health risk via consumption of contaminated chicken offal especially the gizzard and liver as well as restrict chickens from roaming and scavenging for food in dumpsites in order to safeguard the food chain. Further research is required to investigate the dietary intake of heavy metals and possible health risks from other communities where animal free ranging is predominant. This will help to provide consumption advisories to the public about the risks of eating contaminated leaner meat products in an effort to avoid the dangers of high cholesterol.

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Figures and Tables

Table 1: Mean concentrations (mg/kg ww) of heavy metal in different tissues of free range chicken (*Gallus gallus domesticus*), Cattle (*Bos taurus*) and Goat (*Capra hircus*)

		Cr	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Pb**	**Hg**
CL	Mean ± SD	0.064 ± 0.014	2.785 ± 0.440*	91.935 ± 20.228	0.054 ± 0.023	0.019 ± 0.003	88.016 ± 36.349	46.704 ± 5.400	0.006 ± 0.003	0.044 ± 0.013	0.036 ± 0.018	0.001 ± 0.000*
CHL	Mean ± SD	0.077 ± 0.053	3.607 ± 1.198*	305.484 ± 187.966	0.049 ± 0.026	0.018 ± 0.009	4.362 ± 1.017	43.363 ± 13.084	0.069 ± 0.091*	0.291 ± 0.338*	0.171 ± 0.193*	0.034 ± 0.036*
GL	Mean ± SD	0.051 ± 0.030	1.656 ± 0.526*	65.328 ± 27.470	0.033 ± 0.030	0.013 ± 0.005	11.883 ± 8.118	22.731 ± 3.197	0.005 ± 0.005*	0.023 ± 0.009*	0.044 ± 0.032*	0.003 ± 0.002*
CK	Mean ± SD	0.051 ± 0.017	1.109 ± 0.137*	82.850 ± 16.001	0.030 ± 0.008	0.030 ± 0.020	4.187 ± 0.400	30.463 ± 11.204	0.008 ± 0.004*	0.238 ± 0.065	0.052 ± 0.002	0.006 ± 0.003
CHK	Mean ± SD	0.089 ± 0.076	2.468 ± 0.565*	101.521 ± 28.623*	0.049 ± 0.028	0.020 ± 0.004	2.904 ± 0.326	24.700 ± 3.368	0.081 ± 0.073*	0.890 ± 1.035*	0.588 ± 0.703*	0.030 ± 0.031
GK	Mean ± SD	0.060 ± 0.097	0.821 ± 0.302	80.520 ± 22.191*	0.025 ± 0.012	0.028 ± 0.020	2.765 ± 0.298	15.232 ± 2.026	0.008 ± 0.011*	0.123 ± 0.158*	0.072 ± 0.066*	0.019 ± 0.012
CM	Mean ± SD	0.261 ± 0.216	0.163 ± 0.067	28.918 ± 8.863	0.004 ± 0.002	0.023 ± 0.008	0.978 ± 0.312	40.658 ± 20.102	0.007 ± 0.011*	0.001 ± 0.000	0.013 ± 0.010	ND*
CHM	Mean ± SD	0.148 ± 0.166	0.130 ± 0.037*	10.349 ± 5.706	0.004 ± 0.002	0.035 ± 0.032	0.499 ± 0.156	4.430 ± 0.673	0.023 ± 0.018*	0.003 ± 0.003*	0.024 ± 0.029*	0.004 ± 0.003*
GM	Mean ± SD	0.106 ± 0.159	0.187 ± 0.050*	24.792 ± 6.287	0.006 ± 0.006	0.017 ± 0.011	0.825 ± 0.137	24.541 ± 6.737	0.007 ± 0.011*	0.001 ± 0.001*	0.009 ± 0.006*	0.001 ± 0.002*
CHG	Mean ± SD	0.286 ± 0.131	0.547 ± 0.204	80.856 ± 36.112	0.010 ± 0.006	0.049 ± 0.037	3.069 ± 1.412	24.690 ± 4.774	0.047 ± 0.026	0.126 ± 0.111	0.239 ± 0.327	0.003 ± 0.003

CL: Cattle liver; CHL: Chicken Liver; GL: Goat Liver; CK: Cattle kidney; CHK: Chicken kidney; GK: Goat kidney; CM: Cattle muscle; CHM: Chicken muscle; GM: Goat muscle. Letter (*) between columns in the same organ indicates statistical difference (Tukey's test; $p < 0.05$).

ND = Not detectable

Table 2: Estimated Daily Intake (μg/kg bw/day) of Heavy Metals.

Sample Name		**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Pb**	**Hg**
Cattle Liver	Adult	0.161	6.962	229.838	0.135	0.048	220.041	116.760	0.014	0.109	0.089	0.002
	Child	0.214	9.282	306.451	0.180	0.065	293.388	155.681	0.018	0.145	0.119	0.002
Cattle Muscle	Adult	0.653	0.408	72.296	0.011	0.057	2.445	101.644	0.009	0.002	0.034	0
	Child	0.871	0.545	96.394	0.015	0.076	3.260	135.526	0.012	0.003	0.045	0
Cattle Kidney	Adult	0.008	0.185	13.808	0.005	0.005	0.698	5.077	0.001	0.040	0.009	0.001
	Child	0.013	0.296	22.093	0.008	0.008	1.117	8.123	0.002	0.063	0.014	0.002
Chicken Liver	Adult	0.192	9.018	763.710	0.122	0.045	10.905	108.409	0.172	0.728	0.427	0.085
	Child	0.257	12.024	1018.281	0.163	0.060	14.540	144.545	0.229	0.970	0.569	0.113
Chicken Muscle	Adult	0.369	0.326	25.874	0.010	0.088	1.248	11.076	0.057	0.007	0.061	0.009
	Child	0.492	0.434	34.498	0.014	0.117	1.664	14.768	0.076	0.009	0.081	0.013
Chicken Kidney	Adult	0.015	0.411	16.920	0.008	0.003	0.484	4.117	0.014	0.148	0.098	0.005
	Child	0.024	0.658	27.072	0.013	0.005	0.774	6.587	0.022	0.237	0.157	0.008
Chicken Gizzard	Adult	0.716	1.367	202.140	0.026	0.123	7.673	61.725	0.117	0.314	0.596	0.008
	Child	0.954	1.823	269.520	0.034	0.164	10.231	82.300	0.156	0.419	0.795	0.010
Goat Liver	Adult	0.127	4.140	163.320	0.083	0.032	29.707	56.828	0.012	0.058	0.111	0.007
	Child	0.169	5.521	217.760	0.111	0.043	39.610	75.771	0.016	0.077	0.147	0.009
Goat Muscle	Adult	0.264	0.468	61.980	0.014	0.044	2.063	61.353	0.018	0.002	0.022	0.003
	Child	0.352	0.624	82.640	0.018	0.058	2.750	81.804	0.024	0.003	0.030	0.003
Goat Kidney	Adult	0.025	0.342	33.550	0.010	0.012	1.152	6.347	0.003	0.051	0.030	0.008
	Child	0.040	0.547	53.680	0.017	0.019	1.843	10.155	0.005	0.082	0.048	0.012

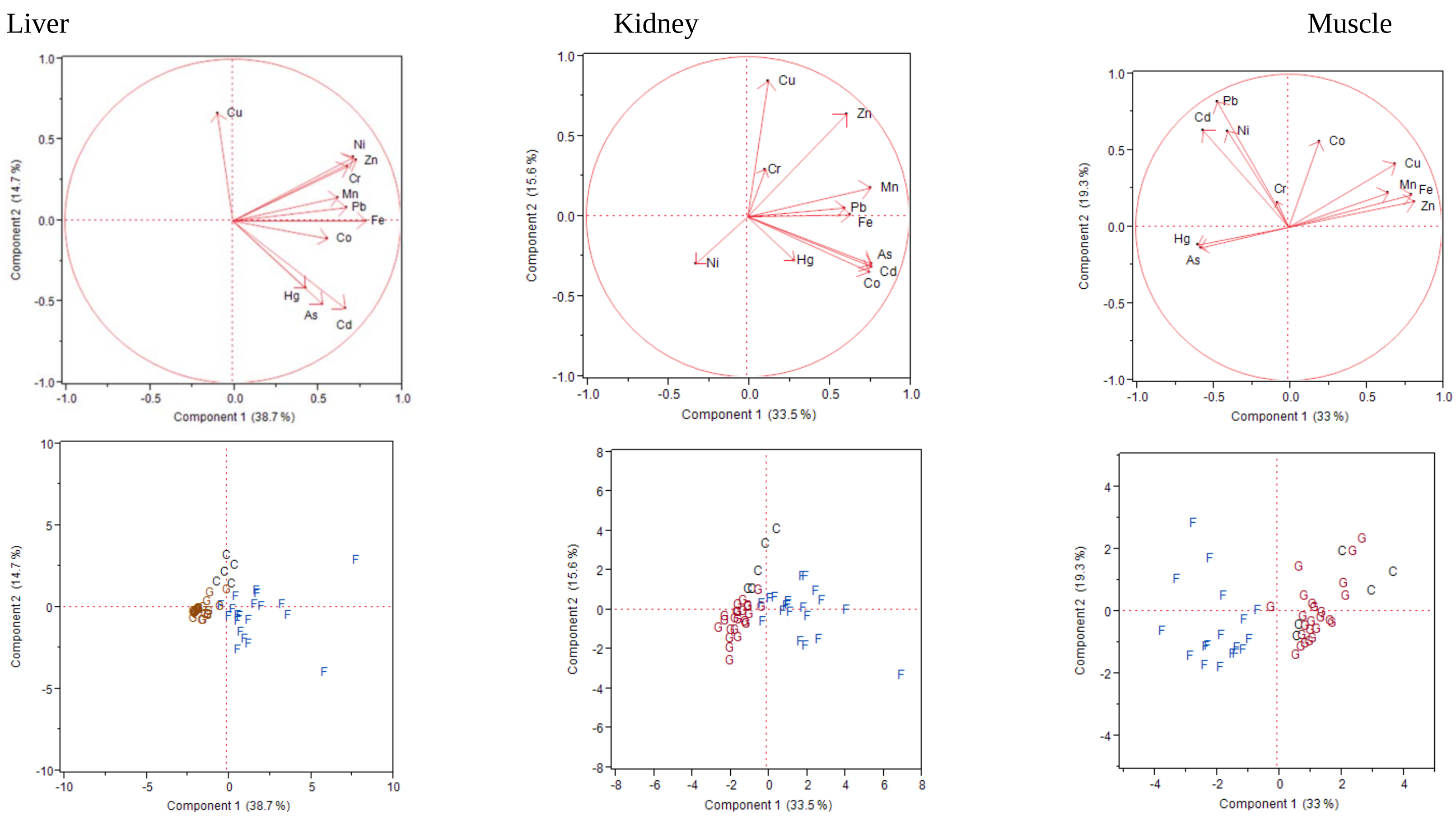
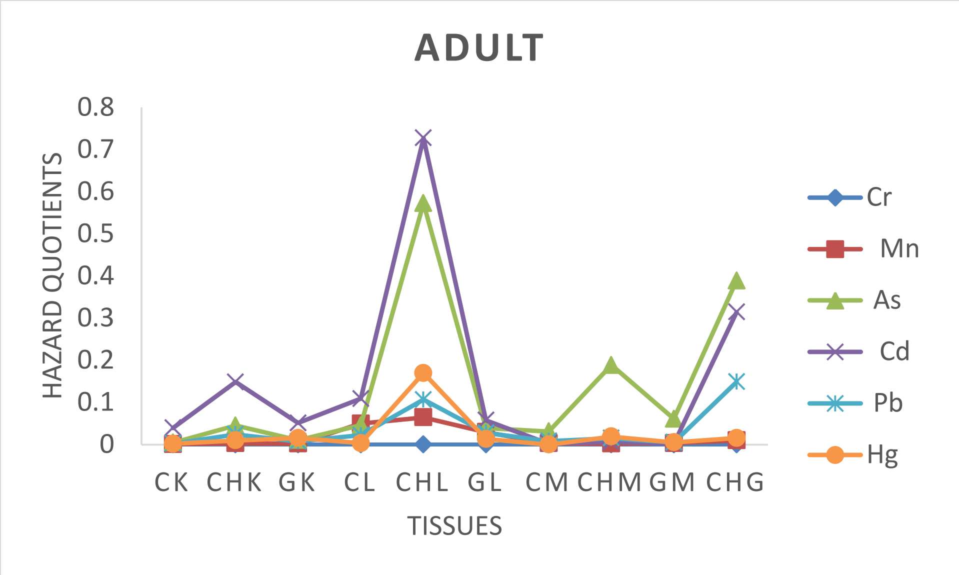


Figure 2: Distribution patterns of heavy metals in tissues of free range chicken (*Gallus gallus domesticus*), Cattle (*Bos taurus*) and Goat (*Capra hircus*) characterized by PCA (C: Cattle; F: Chicken; G: Goat).

Table 3: Rotated component matrix of heavy metal distribution in tissues of Cattle, Chicken and Goat

Metals	**LIVER**			**KIDNEY**			**MUSCLE**		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Cr	0.57295	0.442054	0.3478	0.125716	0.171982	0.586742	0.322429	0.076322	0.560515
Mn	0.746037	0.006849	-0.16617	0.634222	0.451342	-0.13242	0.788645	-0.16787	0.019669
Fe	0.826638	0.164758	-0.30036	0.597652	0.21665	-0.02082	0.860973	-0.23712	-0.11556
Co	0.152608	0.787065	0.167181	0.857003	-0.07441	0.03258	0.563319	0.349646	0.119911
Ni	0.601517	0.471661	0.41585	-0.08658	-0.52952	0.525105	-0.07011	0.754954	0.005269
Cu	0.014404	-0.0496	0.761673	-0.15836	0.839743	0.129631	0.647074	0.065516	-0.49891
Zn	0.843317	0.115863	0.118478	0.385707	0.784202	0.149639	0.702072	-0.23161	-0.39122
As	0.063011	0.784748	-0.27553	0.775946	0.027437	-0.2946	-0.19852	0.069948	0.832405
Cd	0.220286	0.782195	-0.38159	0.823136	-0.0745	-0.10271	-0.1393	0.81524	0.170016
Pb	0.683165	0.19457	-0.12312	0.618564	0.168359	0.287615	-0.03435	0.955143	0.012434
Hg	0.324645	0.200308	-0.54916	0.223473	0.005372	-0.69775	-0.22616	0.055939	0.769395



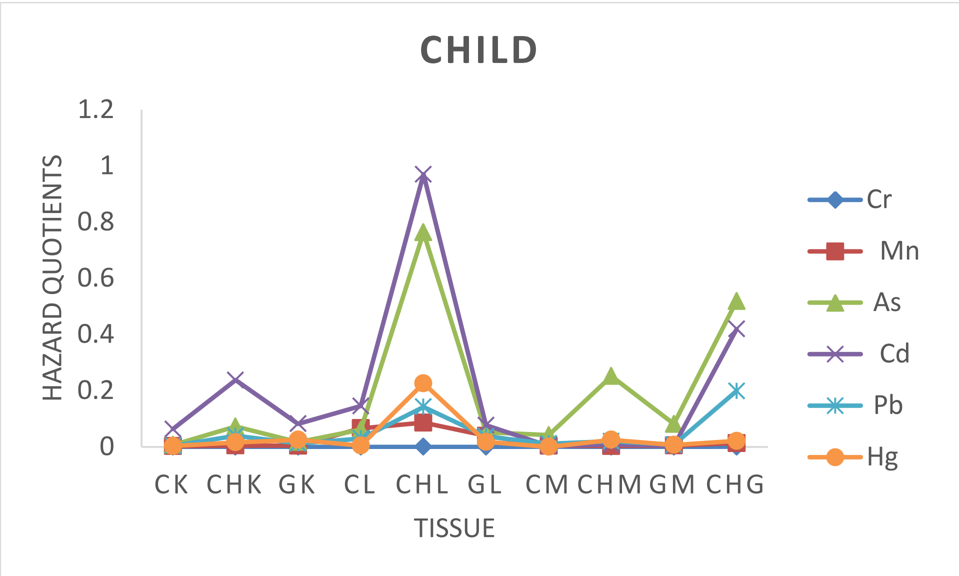


Figure 3: Hazard quotient (THQ) of heavy metals (Cr, Mn, As, Cd, Pb and Hg) in children and adults via consumption of cattle liver CL, chicken liver CHL, goat liver GL, cattle kidney CK, chicken kidney CHK, goat kidney GK, cattle muscle CM, chicken muscle CHM, goat muscle GM, chicken gizzard CHG

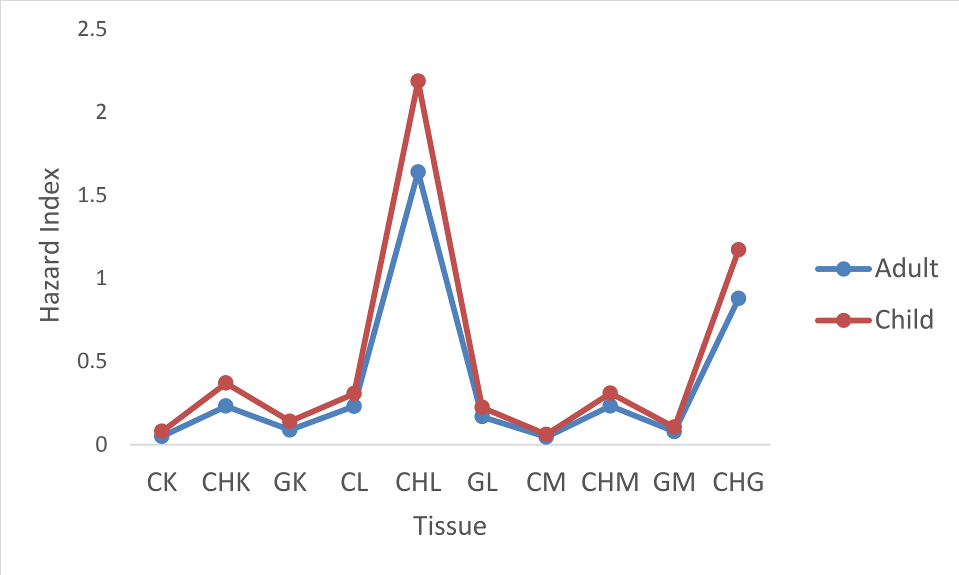


Fig 4: Hazard Index (HI) for children and adults through the consumption of offal and muscle