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# Full Length Research Paper

# Heavy metals distribution and lipid profile in the stomach of cow grazed in Akungba-Akoko, Ondo State, Nigeria

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The distribution of heavy metals: manganese, chromium, cadmium, copper, arsenate, iron and lead together with lipid profiles were investigated in the stomach of cow grazed in Akungba-Akoko region of Ondo State, Nigeria. This sample was analyzed using Atomic Absorption Spectrophotometric method (AAS) with wet digestion method. The results showed that the heavy metals concentrations in the stomach of the cows are: Mn  $(1.04 \pm 0.010 \text{ mg/g})$ , Cr  $(1.85 \pm 0.18 \text{ mg/g})$ , Cd  $(0.055 \pm 0.007 \text{ mg/g})$ , Cu  $(2.525 \pm 0.261 \text{ mg/g})$ , Fe  $(5.59 \pm 0.806 \text{ mg/g})$ , Pb  $(0.065 \pm 0.08 \text{ mg/g})$  and As  $(0.165 \pm 0.021 \text{ mg/g})$ . Also the lipid profile of the stomach of the cow was analyzed using thin layer chromatography. The result obtained was 10.71% phosphatidylcholine, 21.43% phosphatidylethanolamine, 25.0% phosphatidic acid and 42.64% triacylglycerol. The data obtained showed that human consumption of cow stomach may pose a risk of heavy metal toxicosis and hyperlipidemia.

Key words: Heavy metals, lipid profile, stomach, cow, Akungba Akoko, metal toxicity.

#### INTRODUCTION

Heavy metals occur as natural constituents of the earth crust. To a small extent, they enter the body system through food, air and water and bio-accumulate over a period of time (Lenntech, 2004). Contamination of food product by heavy metals is becoming an unavoidable problem these days. Air, soil and water pollution are contributing to the presence of harmful elements such as cadmium, lead, mercury and arsenate in food stuff. The occurrences of heavy metals-enriched ecosystem components firstly arise from rapid industrial growth, advances in agricultural chemicalization, or the urban activities of human beings. These agents have led to metal dispersion in the environment and consequently, impaired health of the population by the ingestion of victuals contaminated by harmful elements (Kudo et al.,

1998). There are a number of different factors that can influence a metals' toxicity, but generally the poisonous effect of heavy metals is a function of a concentration which is consequently both acute and chronic effect. When agricultural soils are polluted, their metals are taken up by plants and consequently accumulate in their tissues (Trueby, 2003). Animals that graze on such contaminated plants and drink from polluted waters as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues and milk (Habashi, 1992; Garbarino et al., 1995; Horsfall and Spiff, 1999; Peplow, 1999). Human are in turn exposed to heavy metals by consuming contaminated plants and animals and this has been known to result in various biochemical disorders (Young, 2005). The frequently reported heavy metals with regard to potential hazard and occurrence in contaminated samples are copper (Cu), lead (Pb), zinc (Zn), nicked (Ni), cobalt (Co), chromium (Cr), iron (Fe), cadmium (Cd) and arsenate (As) (Farlex, 2005).

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**Table 1.** Concentration (mean  $\pm$  SD) and range of heavy metals in stomach of cow in study area (in mg/g).

Heavy metal	Mean ± SD	Range
Mn	$1.040 \pm 0.010$	1.03 - 1.05
Cr	$1.850 \pm 0.180$	1.67 - 2.03
Cd	$0.055 \pm 0.007$	0.05 - 0.06
Cu	$2.525 \pm 0.262$	2.34 - 2.71
As	$0.165 \pm 0.021$	0.15 - 0.18
Fe	$5.590 \pm 0.806$	5.02 - 6.16
Pb	$0.065 \pm 0.086$	0.05 - 0.08

Research findings show that at least 20 million hectares of land in North and South Africa, South America, Middle East, Southern Europe, South West America, Mexico and a significant part of Central and East Asia is irrigated by raw sewage, mainly for cultivation of vegetables. Consequently, this usage ends to soil contamination and heavy metals accumulation both in soil and crops (Carr, 2005; Doyle, 1998).

Cow meat is the major source of protein in most Nigerian diet where people consume the cow flesh, stomach, intestine, both small and large as well as the skin popularly called "ponmon". This study was designed to analyze heavy metals and lipid profile in the stomach of cow grazed in akungba-Akoko, Ondo state.

# **MATERIALS AND METHODS**

#### Study area and sampling

This investigation was carried out within Akungba- Akoko region of Ondo State. At Akungba Akoko abattoir investigated, stomach region of cow (n=5 to 8) was collected for the determination of heavy metals and lipid profile. The samples were collected inside labeled polythene bag.

# Sample preparation

50 g sample of stomach of cow grazed in Akungba community of Ondo state, Nigeria was collected from the abattoir. The sample was collected in a polythene bag and then dried in the oven at  $60^{\circ}\mathrm{C}$  (this allowed for total dehydration of the water content in the sample) for 12 h. After this, the samples was withdrawn and weighed. From the 50 g of stomach sample collected, 2 g was weighted for heavy metal analysis while 10 g was weighed for lipid profile of the sample.

#### Heavy metal quantification

Quantitative analysis of heavy metals was carried out as previously reported by Omotuyi et al. (2006) with slight modifications. Briefly, 2 g samples were accurately weighed in triplicates and ashed in preheated muffle furnace (ACM-82301, ACMAS, India) at 200°C for 30 min, then for 4 h at 480°C. The ash was digested with 10 ml perchloric acid and nitric acid (3:2, v:v). The digestate were made

up to 50 ml using M-Q water (Milli-Q direct, Millipore technologies, Jp). The samples were analyzed by atomic absorption spectrophotometry (932AA, GBC Scientific Equipment, Dandenong, Australia) using appropriate filters.

#### Lipid profile determination

We have previously described the method for determination of lipid profile (Oluyemi et al., 2007). Briefly, 10 g minced samples were dried (20 to 25℃). Samples were suspended in 20 ml chl oroform: methanol (3:2) in capped glass tubes and shaken for at least 6 h at 100 rpm in an orbital shaker at room temperature (25  $\pm$  2°C). The samples were allowed to stand for another 18 h in a slanting position for tissue sedimentation. The chloroform: methanol layer were carefully decanted into a new tube and the solvent were dried off at 30 ± 5℃. The sediments were weighed resuspended in 100 ul fresh chloroform: methanol (3:2). Using capillary tubes, samples were carefully layered on pre-coated TLC plated, and flash dried at 40℃ for 30 s and developed in a TLC tank containing chloroform, methanol and water (6:3:1), respectively. 1.0 mg standard lipids: phosphatidylcholine (L-α-phosphatidylcholine, 61755), phosphatidylethanolamine (L-α-phosphatidylethanolamine, P7943), phosphatidic acid (3-sn-phosphatidic acid, P9511) triacylglycerol (1,2-distearoyl-3-palmitoyl-rac-glycerol, 852-50-5) all purchased from sigma Aldrich (Sigma-Aldrich Japan K.K.), were resuspended in 100 µl chloroform: methanol (3:2) and run along as markers. Lipids were then viewed using UV light and retardation factor (RF) were calculated for samples and standards. Each identified class of phospholipid was scrapped off the plate and in chloroform/ methanol (2:1) and quantified spectrophotometrically using phospho-vanilin method as described by Omotuyi et al. (2011).

# Statistical analysis

Data collected were presented as mean and standard deviation.

#### **RESULTS AND DISCUSSION**

Iron has the highest distribution of 50% among all the heavy metals analyzed (Table 1). The concentration of iron in this study is higher than standard limits recommended by international regulations in Table 2. Although, iron is very important in biological system because of its ability to form complexes and to exist in different oxidation states, it is also present in blood pigment hemoglobin which is able to become reversibly bonded by forming coordinate bonds with oxygen molecular thus allowing hemoglobin to transport oxygen to all part of the body and release it where it is needed (Gupta, 2005). Excess iron in the body can increase the risk of cardiovascular disease (Gupta, 2005). Copper has the second highest distribution of 22% of the total distribution and concentration of 2.34 to 2.71 mg/g which is higher than accepted standards as shown in Table 1. Chromium has a distribution of 16% and concentration of 1.67 to 2.03 mg/g. This concentration is high when compared with the standards in Table 2. On ingestion by human, the chromium supplement interferes with iron absorption which may contribute to irritation and ulcer (Gupta, 2005).

Table 2. Standard limits recommended by international regulations (in mg/g).

Heavy metal	<sup>a</sup> Max. acceptable conc. WHO	<sup>b</sup> Max. acceptable conc. USEPA	<sup>c</sup> Max. acceptable conc. EU
Mn	0.50	0.05	0.05
Cr	0.05	0.10	0.05
Cd	0.03	0.05	0.05
Cu	2.00	1.30	2.00
As	0.01	0.01	0.01
Fe	0.30	0.20	0.30
Pb	0.001	0.02	0.010

Source: a; WHO (2006). b; UNEPA (2004); USEPA (2009). c; Azlan et al. (2012).

**Table 3.** Concentration (mean  $\pm$  SD) and percentage occurrence of lipids in stomach of cow in study area (in mg/g).

Lipid	Amount in mg/g tissue	% occurrence
Phosphatidylcholine	$0.6 \pm 0.033$	10.71
Phosphatidylethanolamine	$1.2 \pm 0.06$	21.43
Phosphatidyl acid	$1.4 \pm 0.01$	25.00
Triacylglycerol	$2.4 \pm 1.20$	42.84

Manganese which is another heavy metal has a concentration of 1.03 to 1.05mg/g and a distribution of 9% of the total distribution. Manganese poisoning is said to occur as a result of high speed drilling of alloys which produces large amounts of Manganese (IV) Oxide dust. Injection of heavily contaminated food has been reported to be associated with chronic and irreversible brain disorder (Ferner, 2001; Duruibe et al., 2007). Arsenic, lead and cadmium have equal distribution of 1% and their concentrations are: 0.15 to 0.18, 0.05 to 0.08 and 0.05 to 0.06 mg/g, respectively (Table 1). The anthropogenic sources of cadmium including industrial emission and the application of fertilizers and sewage sludge to farmland, may lead to contamination of soils and to increased cadmium uptake by crops and vegetables, grown for human and animal consumption. The uptake process of soil cadmium by plant is enhanced by low pH (Jarup et al., 1998; 2000). Continual exposure to this low concentration of cadmium may result in renal dysfunction and obstructive lung disease.

The concentration of lead in this study is  $0.065 \pm 0.086$ . This high concentration may pose a serious adverse effect on human on consumption (Nwude et al., 2011). Ogwuegbu and Muthanga (2005); Ogwuegbu and Ijioma (2003), established that lead poisoning can cause inhibition of the synthesis of hemoglobin, dysfunction of kidneys, joints and reproductive systems, cardiovascular system, acute and chronic damage to the central nervous system and peripheral nervous system.

Considering arsenate which has concentration of 0.15 to 0.18 mg/g (Table 1), though a low concentration but also toxic. The level in soil usually varies due to amount

of pesticide application and waste disposal which can result in higher concentration (Bernard et al., 1992). The low concentration in the stomach of cow in the study area shows there is not much exposure to pesticide application and waste disposed. Hence, if this organ is to be consumed, it must be consumed at a very minimal level and one must avoid frequent intake.

Table 3 shows the lipid distribution in the stomach of cow grazed in Akungba. This shows different percentages of phospholipids in the stomach, phosphatidylcholine 10.71%, phosphatidylethanolamine 21.43%, phosphatidic acid 25.0% and triacylglycerols 42.84%. This is evident because phospholipids make up the cell membranes of plant and animals. They consist of a glycerol backbone with two fatty acids attached, with the third position of glycerol attached to a phosphate group that links an organic base such as choline, ethanolamine, serine, or inositol to the molecule. In the rumen, bacteria largely remove the base group and fatty acids from the phospholipids in dietary ingredient. The higher concentration of triacylglycerols obtained in this study indicate that human intake of cow stomach grazed in the study area may pose a risk of hyperlipidemia (Sudha et al., 2011).

# Conclusion

This study revealed the presence of heavy metals in the stomach of cow grazed in Akungba-Akoko region of Ondo state. The investigated organ exhibit different chemical compositions which is especially evident in the

total amount of heavy metals. The knowledge of the total concentrations of these metals and lipid profile could be considered as a starting point for evaluating the degree of pollution in the environment of Akungba. In addition, the knowledge acquired would assist in ecological risk assessment which is scientifically based on hazard identification, hazard characterization, exposure assessment and risk characterization.

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