Determination of Some Heavy Metals in Kidney, Liver and Muscle of Domestic pig (Sus scrofa domesticus) in Guyuk Metropolis, Adamawa state, Nigeria

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Abstract: **A research conducted on domestic pig (Sus scrofa domesticus) was carried out to determine heavy metal (Pb, Cu, Fe, Ni and Cd) levels in the pork meat (kidney liver and muscle) of domestic pig (S. scrofa domesticus) in Guyuk Metropolis, Adamawa State, Nigeria. Samples of the Sus scrofa domesticus Kidney, Liver, and Muscle (pork meat) were requested and bought from ten (10) different S. scrofa domesticus commercial sellers in Guyuk Metropolis, Adamawa State of Nigeria. The fresh samples were collected and stored in a sample bottle and were transported to Animal Production Laboratory Adamawa State University Mubi for digestion and determination of heavy metals concentration (chemical analysis). Determination of heavy metals was done using Buck Scientific 230 model, Atomic Absorption Spectrophotometer (AAS). The result reveals that, all the heavy metals (Pb, Cu, Fe, Ni and Cd) studied have the highest mean concentration in the Liver, 0.095±0.001mg/g, 0.086±0.001mg/g, 8.165±0.001mg/g, 0.022±0.001mg/g and 0.042±0.001mg/g respectively, followed by muscle 0.085±0.000mg/g, 0.538±0.001mg/g, 3.778±0.001mg/g, 0.011±0.000mg/g and 0.34±0.001mg/g respectively. The least mean concentration of these heavy metals (Pb, Cu, Fe, Ni and Cd) was found in the Kidney with 0.076±0.001mg/g, 0.255±0.001mg/g 0.943±0.001mg/g, 0.010±0.000mg/g and 0.022±0.001mg/g respectively and there was no significant difference of the mean concentrations of Fe, Ni and Cd at (P>0.05) in the organs of the S. scrofa domesticus studied. But there are significant differences of the mean concentration Pb and Cu at (P>0.05) in the organs of the S. scrofa domesticus studied. Female S. scrofa domesticus has higher mean concentration of all the heavy metals than the male S. scrofa domesticus sampled. All the studied heavy metals concentrations were just slightly above the permissible limit set by FAO/WHO. Therefore consumption of pork meat should be minimized to avoid bioaccumulation and biomagnification of these heavy metals. Furthermore, domestic pigs (S. scrofa domesticus) should not be on free range to avoid unnecessary feeding on feeds that contained high amount of heavy metals.**

Keywords: **Domestic pig (Sus scrofa domesticus) heavy metals, Determination, Guyuk, metropolis**

I. INTRODUCTION

Heavy metals occur naturally in the ecosystem with large variations in their concentrations (McDowell et al., 2006; Mohsen and Salisu, 2008; Salwa and Shuharmi-Othman, 2013). They are metallic elements that are toxic and have high density, specific gravity or atomic weight. Heavy metals occur naturally in the soil from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace (<1000mg/g) and rarely toxic (Kabata-Pendias, 2001; Pierzynski et al., 2006). Anthropogenic activities and acceleration of nature's slowly occurring geochemical cycling of materials by man have caused most environments to accumulate one or more of the heavy metals above the defined background value, high enough to cause risks to human health, plant, animals, ecosystems, or other media (D' Amore et al., 2005; Buba et al., 2018[a]).

Intensive animal production systems are on the increase in many regions of the world. Among them pigs are in great importance since is the main livestock in many countries (Gorni et al., 2010). However, increase animal production is not the only aspect in human feed, food safety is also important. Thus, one of the major aspects of food safety is toxic substances such as heavy metals (Maas et al., 2011).

Soil is a long term sink for heavy metals although; they have different mobility and bioavailability (Nicholson et al., 2006). Some metals may bio-accumulate in the food chain, causing human health and environmental concerns (Demirezen and Uruc, 2006; Toor et al., 2007). By nature, animal liver is a natural source of Fe and other essential elements, such as Cu, Mg, Zn and Mn. However, liver might contain higher amounts of heavy metals and other contaminants, which tend to accumulate in liver tissues (Adei and Forson-Adaboh, 2008). Furthermore, heavy metals are not only found in soil and in water by human industrial activity but, according to (Sage, 2007) and Moral et al. (2008), are artificially added in commercial feeds which are often enriched with essential elements such as Cu, Zn, and As in order to promote optimum growth rate and to infuse antimicrobial properties.

Feeds may also contain other nonessential elements such as Cd, Pb, Cr due to their presence in concentrates and supplements and environmental pollution (McBride and Spiers, 2001; Li and Chen, 2005; Sage, 2007). Studies have shown that animals raised in industrial areas have higher concentrations of heavy metals in their internal organs, than animals reared in rural areas (Abou-Arab, 2001). Furthermore, in polluted areas with rust scrub metals in Nigeria where dairy cows were raised, concentration of Pb in blood, milk and animal wastes, increased significantly compared with cows raised in uncontaminated areas of the country (Ogundiran et al., 2012).

There are scanty studies on the content of heavy metals in companion animal (Kozak et al., 2002). Environmental pollution by heavy metals is ubiquitous; this is due to both the natural abundance of metals within earth's crust and human activities (Langner et al., 2011; Markert et al., 2011). Some of them are of great toxicological concern and have a wide range of toxic effects in both humans and animals. Toxic effects usually associated with chronic exposure include; mutagenicity, carcinogenicity, teratogenicity, immuno-suppression, poor body condition and impaired reproduction (Beyersmann and Hartwig, 2008; Garcia-Leston et al., 2010; Lehmann et al., 2011; Reif, 2011). However, information about the possible contamination of free range of domestic pig (S. scrofa domesticus) which is highly consumed by human in Guyuk Local Government Area of Adamawa State is not known. The domestic pig (S. scrofa domesticus) are not restricted, they are exposed to various area of possible accumulation of waste water and feeds from various sources hence the purpose of this research. This research work was carried out mainly to determine the concentration level of heavy metals (lead, copper, iron, nickel and cadmium) in liver, muscles and kidney of domestic pig (S. scrofa domesticus) and to compare by sex the concentration level of heavy metals in domestic pig (S. scrofa domesticus) in Guyuk Metropolis Adamawa State, Nigeria. This study will give knowledge to understand the status of these heavy metals concentration level (lead, copper, iron, nickel and cadmium) and subsequence transfer of these heavy metals in food chain and to serve as baseline of the heavy metals accumulation of other companion animals in the study area.

II. MATERIAL AND METHODS

Sampling area

Sampling area and sampling site was from Guyuk metropolis Adamawa State, Nigeria. Guyuk Local Government Area is located at an elevation of 198 meters above sea level. Its coordinates are 9°54'0'' N and 11°58'60'' E. Adamawa State is located in the North Eastern part of Nigeria, and lies between latitudes 7° and 11° N and between longitudes 11° and 14° E. Adamawa State is on an altitude of 185 meter above Sea level and covers a land area of about 39, 741km^2 , (Adebayo et al., 2012).

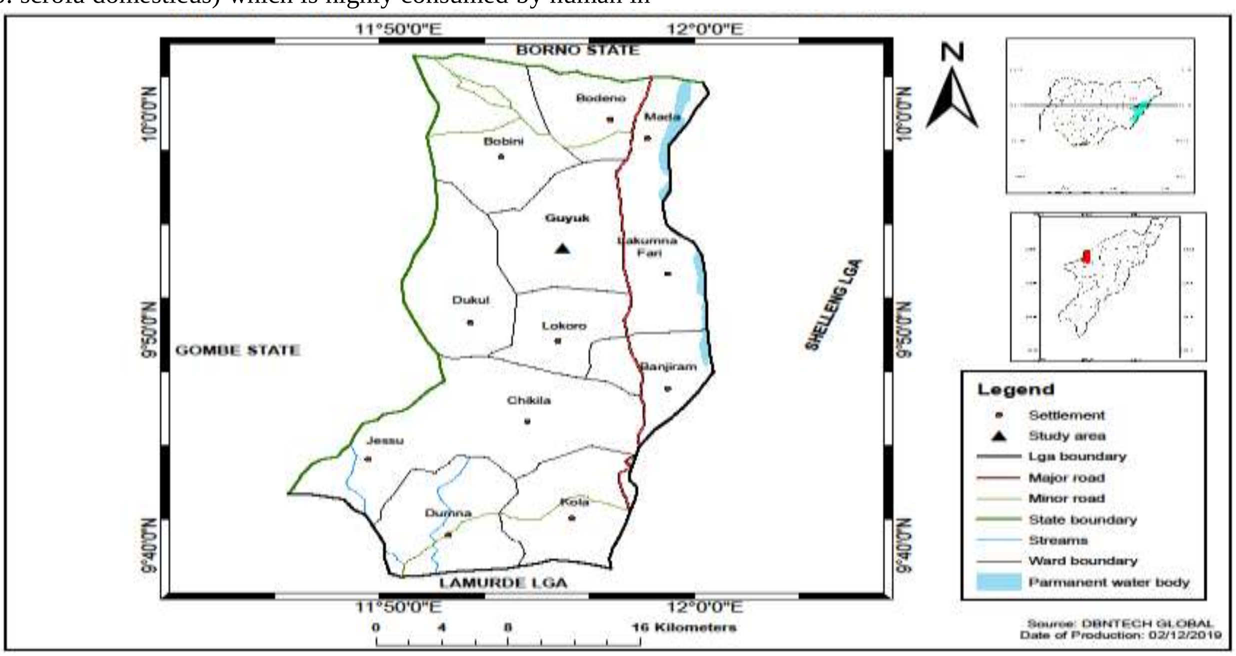

Fig: 1 The Map Guyuk Local Government Area Of Adamawa State, Nigeria Showing The Study Area.

III. SAMPLE COLLECTION

Pork meat [parts of the domestic pig (Sus scrofa domesticus)] Kidney, Liver, and Muscle were bought from ten (10) different demostic pig (Sus scrofa domesticus) commercial sellers in Guyuk Metropolis, Guyuk Local Government Adamawa State of Nigeria. The fresh samples were collected and stored in a sample bottle and transported to Animal Production Laboratory Adamawa State University Mubi for chemical analysis.

Sample preparation and Determination of heavy metals

The organs samples were homogenized each separately and 10g of fresh homogenized samples was weighed into porcelain dishes and evaporates to dryness in an oven at 105°C. The ground samples of Sus scrofa domesticus were transferred to a porcelain basin and put into a muffle furnace and the temperature was increased gradually until 550°C was reached. The samples were digested with tri-acid mixture (HNO_3: HCO_4: H_2SO_4) in the ratio of 10:4:1, respectively at a rate of 10ml per 10g of sample and were placed on a hot plate at 100°C temperature. Digestion was allowed to continue until the liquor became clear. All the digested liquor was filtered through Whatman 541 filter paper and diluted with 25mls of distilled water as in Akan et al., (2012). Determination of the heavy metals was done directly on each final solution using a Buck Scientific 230A Model, Atomic Absorption Spectrophotometer (AAS). The heavy metals (Pb, Fe, Cu Ni and Cd) concentration was quantify from the calibration curve of the standard. Values obtained were expressed in milligram per gram (mg/g) following standard of (AOAC, 2010; APHA, 2017).

Data analysis

Data obtained were presented as mean and standard error mean and were subjected to one way analysis of variance (ANOVA) followed by Duncan's multiple comparison test. And student T- test was used to compare the mean concentration of heavy metals between male and female. P>0.05 was considered as there was no significant difference, using SPSS (version 20) to assess whether heavy metals varied significantly between the organs (kidney, liver, and muscle) and also by sex of domestic pig (Sus scrofa domesticus).

IV. RESULTS

The result of this research work shows the mean concentrations of all the studied heavy metals in kidney, liver, and muscles of domestic pig (S. scrofa domesticus). In Table 1. Lead has the highest mean concentration (0.095±0.001mg/g) in the Liver, followed by muscle (0.085±0.000mg/g). The least mean concentration of lead was found in the Kidney with (0.076±0.001mg/g) and the differences of the mean concentrations were not significant at (P>0.05).

For Iron, the result shows that Liver had the highest mean concentration of 8.165±0.001mg/g, followed by muscles with mean concentration of 3.778±0.001mg/g. The least mean concentration of Iron was found in the kidney with a value of 0.943±0.001mg/g, but there was significant difference (P>0.05). The result for Cadmium, shows that the Liver of the Pig sample had the highest mean concentration (0.042±0.001mg/g), followed by the muscle (0.34±0.001mg/g). The domestic pig Kidney has the least mean concentration of 0.022±0.001mg/g, and the differences was not significant (P>0.05).

Nickel has the highest mean concentration of (0.022±0.001mg/g) in the Liver of the domestic pig, followed by muscle with mean concentration of (0.011±0.000mg/g). The least mean concentration of Nickel was found in the pig Kidney with a value of (0.010±0.000mg/g) and the difference were not significant at (P>0.05). The highest concentration of Copper was observed in the Liver of the domestic pig sample with the mean concentration of (0.086±0.001mg/g), followed by the muscles with mean concentration of (0.054±0.001mg/g). The least mean concentration of copper was found in the Kidney with (0.255±0.001mg/g) and the differences were significant at (P<0.05).

Table 1: Mean Concentration of Heavy Metals in Kidney, Liver and Muscle of domestic Pig of both sexes (mg/g)

Organs			Heavy metals		
	Lead	Iron	Cadmium	Nickel	Copper
Kidney	0.076 ± 0.000^{a}	0.943 ± 0.001^{a}	0.022 ± 0.001^{c}	0.010 ± 0.000^{a}	0.255 ± 0.001^{a}
Liver	0.095 ± 0.000^{a}	8.165 ± 0.001^{c}	0.042 ± 0.001^{c}	0.022 ± 0.001^{a}	0.086 ± 0.001^{c}
Muscles	0.085 ± 0.000^{a} ns	3.778 ± 0.001^{b} *	0.034 ± 0.001^{c} ns	0.011 ± 0.000^{a} ns	0.054 ± 0.001^{b} *

P>0.05

NB: Values with the same superscripts in each row are not significantly different from each other at (P>0.05) using ANOVA and DMRT for mean separation. Values are expressed as mean ±. P<0.05 was considered significant.

Key: * = Significant

Table 2 and Table 3, shows that, all the heavy metals studied have high mean concentration in the liver, followed by the muscles and the least mean concentration was observed in the kidney of male and female domestic pigs (S. scrofa domesticus).

Table 2: Mean concentration of Heavy Metals in Kidney, Liver, and Muscle of male domestic Pigs (mg/g)

Organs			Heavy metals		
	Lead	Iron	Cadmium	Nickel	Copper
Kidney	0.072 ± 0.000^{a}	1.543 ± 0.001^{a}	0.024 ± 0.001^{c}	0.010 ± 0.000^{a}	0.253 ± 0.001^{a}
Liver	0.085 ± 0.003^{a}	7.665 ± 0.001^{c}	0.032 ± 0.001^{c}	0.012 ± 0.001^{a}	0.066 ± 0.003^{c}
Muscles	0.073 ± 0.002^{a} ns	5.474 ± 0.001^{b} *	0.034 ± 0.002^{c} ns	0.014 ± 0.001^{a} ns	0.052 ± 0.001^{b} *

P>0.05

NB: Values with the same superscripts in each row are not significantly different from each other at (P>0.05) using ANOVA and DMRT for mean separation. Values are expressed as mean ±. P>0.05 was considered no significant.

Key: * = Significant ns = No significant

Table 3: Mean concentration of Heavy Metals in Kidney, Liver, and Muscle of female domestic Pigs (mg/g)

Organs			Heavy metals		
	Lead	Iron	Cadmium	Nickel	Copper
Kidney	0.076±0.003[a]	2.943±0.001[a]	0.022±0.001[c]	0.010±0.000[a]	0.255±0.001[a]
Liver	0.095±0.001[a]	8.165±0.002[c]	0.042±0.002[c]	0.022±0.001[a]	0.086±0.003[c]
Muscles	0.085±0.003[a] ns	5.778±0.002[b] *	0.034±0.002[c] ns	0.011±0.000[a] ns	0.054±0.001[b] *

P>0.05

NB: Values with the same superscripts in each row are not significantly different from each other at (P>0.05) using ANOVA and DMRT for mean separation. Values are expressed as mean ±. P>0.05 was considered no significant.

Key: * = Significant

ns = No significant

Comparison between the levels of mean concentration of heavy metals in male and female domestic pigs (S. scrofa domesticus) sampled in Guyuk Local Government Area of Adamawa State, Nigeria, shows that, of all the heavy metals female has the highest level of mean concentration than the male domestic pig (S. scrofa domesticus) as seen in table 4.

Table 4: Mean Concentration of Some Heavy Metals in Male and Female domestic pig (Sus scrofa domesticus) (mg/g)

Metals	Male S. scrofa domesticus	Female S. scrofa domesticus
Pb	0.077±0.002[a]	0.085±0.003[c] *
Fe	4.894±0.001[c]	5.629±0.001[b] *
Cd	0.030±0.004[a]	0.033±0.001[a] ns
NI	0.012±0.003[b]	0.014±0.001[b] ns
Cu	0.124±0.001[c]	0.132±0.001[a] *

NB: Means with the same superscripts in each column are not significantly different at (p> 0.05)

Key: ns = not significant

* = significant

ns = No significant

V. DISCUSSION

The result of this study shows a significant variability in the accumulation of heavy metals in the different organs (liver, kidney, and muscles) of domestic pig (S. scrofa domesticus) in Guyuk Metropolis, Adamawa State, Nigeria, which is principally dependent on the bioavailable metal concentration in their environment as stated by Peakall and Burger, (2003) and Marchovecchio, (2014). That the higher the bioavailability of heavy metals in an environment the higher the chance of absorption into the animals that lives, feed and drink in that environment. The findings of this study indicate that the highest mean concentration of Lead (Pb) (0.095±0.000mg/g) was in the liver of the domestic pig followed by the muscles (0.085±0.000mg/g), the least concentration of lead (0.076±0.001mg/g) was in the kidney. Therefore, the mean concentration of lead in this study has exceeded the concentration level of lead (0.035mg/g) for ruminants as reported by Radostitis et al., 2000. The result of this study is also in line with the findings of (Behnaz et al., 2016), who reported higher mean concentration of lead in the liver of slaughtered cattle (21.1±3.30mg/kg) and kidney (28.10±3.39mg/kg), and also in the liver of sheep (17.05±5.17mg/kg) and kidney (14.34±4.62mg/kg) from a mining region in the west of Iran. Lead is considered as the most common form of poisoning in farm animals.

The Liver of domestic pig (S. scrofa domesticus) has the highest mean concentration of iron (8.165±0.001mg/g), followed by muscles with (3.778±0.001mg/g), the least concentration of iron was observed in the Kidney with a value of (0.943±0.001mg/g). The result of Iron mean concentration in the organs of the domestic pig (S. scrofa domesticus) in this study, does not exceed the permissible limit for Iron in food, which is generally 30-150mg/kg as stated by (Demirezen and Uric, 2006). The findings of this study does not agree with the result of (Behnaz et al., 2006), who reported the value of Iron concentration (101.99-199.28mg/kg) in the Liver of cattle and sheep respectively, which has exceeded the permissible limit for Iron in food. This would be due to its bioavailability of iron in the environment and also the type of animals involved in the study.

Cadmium was found in the liver of the domestic pig (Sus scrofa domesticus) with the highest mean concentration of (0.042±0.001mg/g), followed by the muscle with 0.034±0.001mg/g), the least mean concentration was found in the kidney with a value of (0.022±0.001mg/g). The result of Cadmium concentration in the organs of the domestic pig (Sus scrofa domesticus) in this study is higher than the permissible limit for Cadmium (0.5ppm) as set by (FAO/WHO, 2000). The result of this study does not agree with the result of Akan et al., 2010, who reported lower concentration of Cadmium in the kidney of sheep (0.76±0.15µg/g) and also lower concentration of cadmium in the meat of cow (0.07µg/g) from kasuwan shanu market in Maiduguri Metropolis, Borno State, Nigeria. This may be due to the environmental contamination of these metals in Guyuk Metropolis, Adamawa State, Nigeria than Maiduguri Metropolis, Borno State, Nigeria. Cadmium accumulates in the human body affecting negatively several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system (Castro-González & Méndez-Armenta, 2008). Other damages that have been observed include reproductive and development, hepatic, haematological and immunological effects (Apostoli and Catalani, 2011; ATSDR, 2011).

The liver of the domestic pig (S. scrofa domesticus) has the highest mean concentration of Ni (0.022±0.001mg/g), followed by muscle with (0.011±0.000mg/g), the least concentration of nickel was observed in the kidney of domestic pig (sus scrofa domesticus) with a value of (0.010±0.000mg/g). The overall mean concentration of nickel in this study was higher than the tolerable limit (0.5µg/kg) set by World Health Organization (WHO). The finding of nickel concentration in the organs of the domestic pig in this study has agreed with the result of (Nasser et al., 2013), who reported higher (0.025-0.275mg/kg) concentration of nickel in the organs of buffalo and sheep in Kohat market Parkistan. The major source of nickel for humans is food and uptake from natural sources, as well as food processing (Nas-NRC 1975; Akan et al., 2012; Buba et al., 2018[b]). Increased incidence of cancer of the lungs and nasal cavity caused by high intake of nickel in animals has also been reported by (Anonymous, 2013; Buba et al., 2018).

Liver has the highest mean concentration of copper (Cu), followed by kidney, the least concentration was found in the muscle of the domestic pig (S. scrofa domesticus). A significantly higher level of copper (Cu) in the Muscle than in the Liver and Intestine had been observed by (Torres et al., 2010), which is not in line with the finding of this study. This may be due to the bioavailability of copper in the study area. Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin.

The results of this study is similar with the findings of Nesta et al. (2016), who stated that the average Cd levels in the liver (0.12 mg/g dw) and kidneys (0.65 mg/g dw) of females were two times higher than in male liver (0.07 mg/g dw) and kidneys (0.29 mg/g dw), respectively in Tarkwa, Ghana. Absorption of some heavy metals is through the gastrointestinal tract (GIT), however this can be affected by several factors, such as age, sex, nutritional status, and preceding metal burden. Among these, young age, iron deficiency, and being female are reported to accelerate the absorption of metals through the GIT in both humans and animals (Nesta et al. 2016).These could be the reasons why concentrations of heavy metals were higher in females than males. The finding of this study is also in line with (Damek-Poprawa and Sawicka-Kapusta, 2004), who reported that bio-accumulation of heavy metals in animals varies according to their sex, size and age. Although, the ages and size of S. scrofa domesticus in this study was not determined, the results of sex differences in the accumulation of Cd and Ni in this study showed no statistical variation ($P > 0.05$). However, the level of Pb, Fe and Cu are significantly different in the sexes of S. scrofa domesticus sampled.

VI. CONCLUSION

In conclusion, the results obtained in this research work in the liver, kidney, and muscle of domestic pig (S. scrofa domesticus) sampled shows that the mean concentration of Lead, Iron, Cadmium, Nickel, and Copper were within the permissible limit set by FAO/WHO. It also shows that female domestic pigs have higher mean concentration compared with the male domestic pigs. Therefore, the researcher recommends that consumption of the female domestic pig (S. scrofa domesticus) should be minimize in order to avoid bioaccumulation of this heavy metals in human body. Domestic pig (S. scrofa domesticus) should not be allowed to be on free range to avoid unnecessary feeding on feeds that contained high amount of heavy metals because of its natural curiousty, licking habits and lack of oral discrimination.

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