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Original Article

Assessment of lead (Pb) residues in organs and muscles of slaughtered pigs at Nsukka and Environs in Enugu state, Nigeria

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ABSTRACT

Objective: Heavy metals are serious environmental pollutants and their uptake and accumulation in the ecosystem, beyond safe limits, would cause direct consequences to food chain and ultimately to man. The present study was undertaken to ascertain the presence and levels of lead (Pb) in muscles and organs of slaughtered pigs at Nsukka agricultural zone, Nigeria.
Materials and methods: From a total of 160 slaughtered pigs, liver, kidney and muscle of each pig were collected and processed for the detection of lead residue using Atomic Absorption Spectrophotometer.
Results: A prevalence rate of 81.25% was recorded for Pb residue in Nsukka agricultural zone. There is a strong association ($P<0.0001$) between occurrence of Pb residue and the source of samples. Mean concentrations of 0.0758 mg/kg, 0.1050 mg/kg and 0.0985 mg/kg were recorded in kidney, mean concentrations of 0.1676 mg/kg, 0.1235 mg/kg and 0.1955 mg/kg were recorded in liver and mean concentrations of 0.0598 mg/kg, 0.0870 mg/kg and 0.1020 mg/kg in muscle samples in Nsukka, orba and Obollo slaughter houses respectively. There is no significant ($P<0.05$) difference between the mean concentrations of the Pb in the liver, kidney and muscle samples (Nsukka, Orba and Obollo) and its maximum permissible levels (MPL).
Conclusion: More than half of the samples were positive for Pb and few samples that exceeded the MPL may pose human health threat to pork consumers in the study area.

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KEYWORDS

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INTRODUCTION

Heavy metals are naturally occurring elements in the earth's crust, and thus exposure to natural sources is inevitable. The metals are found in various concentrations in the soil, sediments, surface and groundwaters, air and even in tissues and organs of food animals. The grazing of food animals on contaminated soil has given rise to higher levels of heavy metals in beef and mutton (Sabir et al., 2003). Ingestion of the contaminants by animals causes deposition of residues in meat. Illegal mining of ores, painting of animals' houses, and methods of processing slaughtered pigs have been incriminated in habitual contamination of animals and their products with heavy metals (Bala et al., 2012a, b; Felix et al., 2016). Indiscriminate dumping of waste materials and sewage water on land gradually increases the concentration of heavy metals in the soil which are increasingly taken up by the plants and vegetables. The contaminated fruits and vegetables find their way into the food chain causing severe health hazards to both animals and humans (Haiyan and Stuanes, 2003). Pigs may drink water from ponds, streams, rivers and other possible contaminated water sources (Felix et al., 2016).

Lead is a naturally occurring bluish-grey metal found in small amounts in the earth's crust (ATSDR, 2007). It is a ubiquitous element that is found in rocks, soil, plants, animals and human beings however, it naturally occurs quite in a low level (Griffith, 2002). For centuries, lead has been mined, used in industry and in household products such as petrol, cigarettes, paint, ceramic glazes, smelters, televisions, pesticides, computer monitors, batteries, explosives, pipes and toys (Kalu et al., 2014). Long-term exposure of adults to lead can result in decreased performance in some tests that measure functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anemia (ATSDR, 2007). Exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death. High level exposure to lead in men and women may damage the organs responsible for sperm production and cause miscarriage respectively. The risk associated with the exposure to heavy metals present in food products had aroused widespread concern for human health (Santhi et al., 2008).

Different researchers have reported the instances of contamination of heavy metals in meat products during processing (Brito et al., 2005; Santhi et al., 2008). Heavy metals residues were found in meat and meat product of food animals fed with contaminated feed and reared in proximity to polluted environments (Korenekova et al., 2002; Sabir et al., 2003; Miranda et al., 2005).

The above statements show how heavy metals posed serious health hazard to general public. The main objective of this study is to determine the presence, prevalence and concentration of lead residues in organs and muscles of slaughtered pigs in the study area.

MATERIALS AND METHODS

Study area: The study was carried out in Nsukka agricultural zone of Enugu State, South East Nigeria. Nsukka agricultural zone has three major slaughter houses located at: Nsukka urban with map coordinates of 6°51'24"N and 7°23'45"E. Orba with a map coordinates of 6°51'0"N and 7°27'0"E and Obollo-Afor with coordinates of 6°N and 7°E (**Figure 1**). Nsukka has a total land area of about 17.5 sq mi (45.38 km²), and has an elevation of 1,810ft (522 m) with a population of 309, 633 (NPC, 2006).

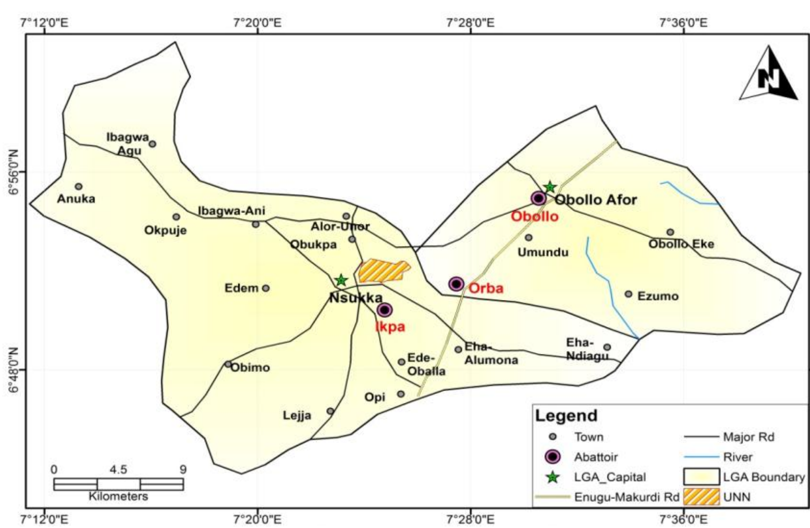

Figure 1: Map of Nsukka agricultural zone showing major slaughter houses

Study Design: The research work was a four month cross sectional survey and laboratory analysis of post slaughter matrix samples from slaughtered pigs, to determine the presence and concentration of lead (Felix et al., 2016).

Sampling technique and sample collection: One (Nsukka) out of the three agricultural zones in Enugu State was randomly selected. The three major slaughter houses (Nsukka urban, Orba and Obollo Afor) were purposively selected. Stratified random sampling was used to select pigs from each slaughter house assigning them into female and male sex strata and systematic random sampling was used to select 1 in 3 pigs slaughtered from each group, twice a week for four months.

A total of 480 fresh samples of liver, kidney and muscle from 160 slaughtered pigs were collected between the months of June 2014 and September 2014. Eighty (80) pigs were sampled from Nsukka urban since it has a higher slaughter capacity than the other two. Forty (40) pigs each were sampled from Orba and Obollo-afor slaughter houses since they have the same slaughter capacity. Age was determined using teeth eruption and wearing. About 50g each of liver and muscle samples and a whole kidney of each selected slaughter pig was packed in sterile polythene bags, labeled and sent to Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka for freezing. The frozen samples were transported in a cold chain to Springboard Research laboratory, Awka Anambra State, Nigeria, for chemical analysis. Information on the method of processing and the type of materials used was collected by observation and pictures were taken. The experiments were conducted with the permission of the Institution's Ethics Committee.

Sample processing

Digestion of sample (dry digestion): Liver, kidney and muscle samples were dried in the oven at 45°C. After drying, individual sample was crushed into fine powder using mortar and pestle, and 1.0 g of the fine powdered sample was weighed into porcelain crucible and ignited in a muffle furnace at 500°C for 6 to 8 h. The samples were then removed from the furnace and allowed to cool in desiccators, and weighed again. 5 cm cube of 1 M Trioxonitrate (V) acid (HNO_3) solution was added to the left-over ash and evaporated to dryness on a hot plate and returned to the furnace for re-heating at 400°C for 15-20 min until perfect grayish-white ash was obtained. The samples were then allowed to cool in desiccators. 15ml (cm^3) hydrochloric acid (HCL) was then added to the ash to dissolve it and the solution was filtered into 100 cm^3 volumetric flask. The volume was made to 100cm^3 with distilled water.

Analyses: Lead (Pb) residues were tested for in the digested liver, kidney and muscle under specified condition using Atomic Absorption Spectrometer (AAS). The procedure was done according to Felix et al. (2016).

- *Stock Standard Solution:* Lead**,** 1000 mg/l. Dissolve 1,000 g of Lead metal in a minimum volume of (1+1) HCL. Dilute to 1 liter with 1% (v/v) HCL.
- *Light Sources:* Hollow cathode lamps were used for lead.

Data analysis and presentation: The data generated from the study were statistically analyzed using both SPSS version 17 and GraphPad Prism Statistical software version 5.02 (www.graphpad.com). Gaussian distribution of data sets was tested for, using D'agostino Omnibus Normality test before choosing the most appropriate statistical tests. Chi square analysis was used to determine if there is an association between the occurrence of lead residue and the source of samples and type of organ. Analysis of variance and post hoc test were performed to determine if there is significance difference in the mean concentrations of lead among various age groups. Wilcoxon signed rank test was used to determine if there is a significant difference in the mean concentration of lead in the organs and their specific Maximum Permissible Limit (MPL). The alpha value of significance was set at the Probability level of <0.05.

RESULTS AND DISCUSSION

This study recorded a high prevalence rate of 81.25% which is indicative of high exposure of pig consumers to lead residue. Out of a total of 160 pigs sampled, 130 (81.25%) were positive while 30 (18.75%) were negative for lead residue and from 480 organs sampled (160 each of liver, kidney and muscle from the 160 pigs), 332 (69.17%) were positive while 148 (30.83%) were negative for lead residue (**Table 1**).

The prevalence recorded in this study was similar to the report of Iwegbue (2008), who recorded a prevalence rate of 75% in slaughter cattle at southern Nigeria. However, our findings slightly differed from Bala et al. (2012a) who showed a 100 % prevalence of Pb residue in all the liver and kidney samples collected from the slaughtered pigs at Sabo-Wakama Market of Akun Development Area of Nasarawa State. The parity between the prevalence of lead residue in pigs from Nsukka urban abattoir (75%) and other slaughter houses (Orba and

Table 1: Prevalence of lead residue in slaughtered pigs in Nsukka Agricultural zone

Status	No of Pigs (%)	No of Organ types (%)			Total
		Liver	Kidney	Muscle	
Positive	130(81.25)	110(68.75)	110(68.75)	112(70)	332(69.17)
Negative	30(18.75)	50(31.25)	50(31.25)	48(30)	148(30.83)
Total	160	160	160	160	480

Table 2: The number (%) of organs of slaughter pigs with mean concentration above MPL

Sample type	No sampled	Maximum Permissible Limit (MPL)	
		No (%) below	No (%) above
Kidney	160	158 (98.75)	2 (1.25)
Liver	160	148 (92.5)	12(7.5)
Muscle	160	116 (72.5)	44(27.5)
Total	480	422 (87.92)	58(12.08)

Table 3: Mean and SEM organ concentration of lead in slaughtered pigs according to Age range.

Age range of pigs	Organ mean and standard error of the mean (SEM) concentration (mg/kg)		
	Kidney	Liver	Muscle
0-1	0.1621 ± 0.0317^{a}	0.3386 ± 0.0885^{a}	0.1467 ± 0.0248^{a}
2-3	0.0973 ± 0.0145^{b}	0.1346 ± 0.0304^{b}	0.0619 ± 0.0162^{b}
4-5	0.0222 ± 0.0090^{c}	0.0472 ± 0.0180^{b}	0.0458 ± 0.0143^{b}
≥6	0.0467 ± 0.0127^{d}	0.0946 ± 0.0590^{b}	0.0375 ± 0.0175^{b}

Values within same column with different superscripts are statistically different at P<0.05. SEM: Standard Error of mean

Obollo Afor) in this study could be likened to the fact that Nsukka is located in urban area and students are taken to those abattoirs for meat inspection and teaching indirectly creating awareness, because of their proximity to the University. Orba and Obollo Afor slaughter houses are interiorly located and so are exposed to more mundane singeing practices and higher rate of exposure to cadmium due to the processing materials or methods (fueling of wood with kerosene, fuel, plastic, metal stripes and tyre) used in singeing the slaughtered pigs as also reported by Kalu et al. (2014) and Felix et al. (2016). Also, awareness of the dangers of such practices is low as such exposure to lead due to such practices may be due to ignorance on the part of the butchers, hence the 85% and 90% prevalence recorded for Orba and Obollo respectively, with a strong association between the occurrence of lead residue and location/source of samples. Sixty (75%) out of the 80 pigs sampled from Nsukka slaughterhouse were positive for lead residue, 34 (85%) of 40 pigs in Orba and 36 (90%) of 40 in Obollo were positive for lead residue (**Figure 2**). There is no association (P=0.669) between occurrence of lead and the source of samples.

In **Figure 3**, the presence of lead was recorded in 110 (68.75%) of liver, 110 (68.75%) of kidney and 112 (70%) of muscle samples. There is no association (P=0.967)

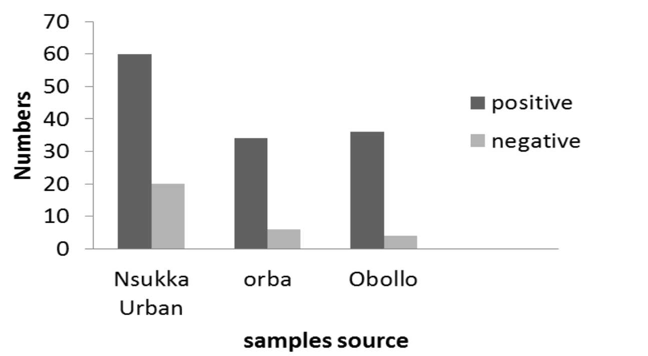

Figure 2: Source/Location distribution of lead in slaughtered pigs

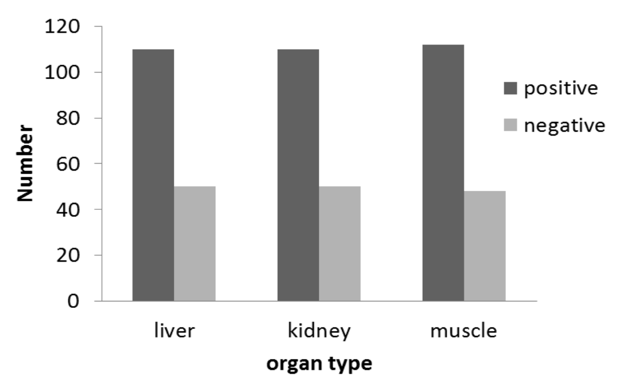

Figure 3: Organ distributions of lead

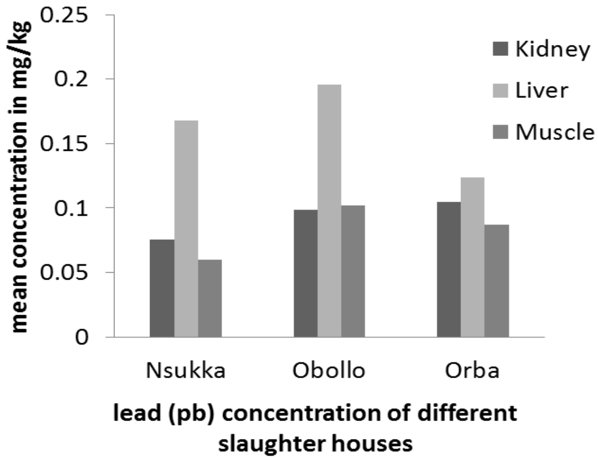

Figure 4: Levels of lead concentrations in kidney, liver and muscles of slaughtered pigs in Nsukka, Orba and Obollo slaughter houses, Enugu State

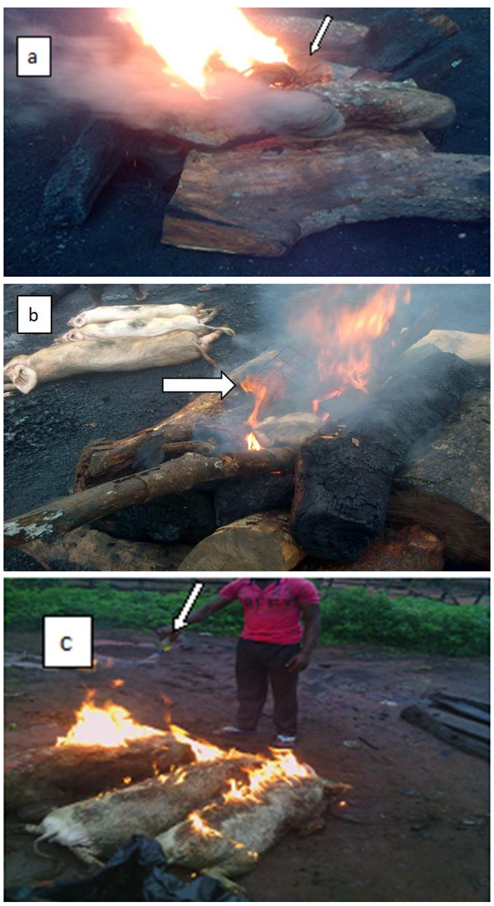

Figure 5a-c: Direct use of tyre, metal stripes and petrol in scorching the skin of slaughtered pigs.

between occurrence of lead and the type of organ. Although it has been reported that lead residue accumulates more in kidney and liver (Hiba, 2011) as also detected in this work owing to the fact that kidney and liver are organs of biotransformation and detoxification, but no association was found between the type of tissue and the occurrence of lead residues with 68.75%, 68.75% and 70% prevalence for kidney, liver and muscle respectively. The non association could be likened to the singeing practices which makes accumulation of lead in the muscle almost as high as in the internal organs (kidney and liver). Accumulation in internal organs (kidney and liver) occur due to oral exposure (consumption of lead contaminated food and water). Animals, especially free range pigs are exposed to heavy metals in our local environment through scavenging in open waste or refuse dumps, and polluted drinking water (Obiri-Danso et al., 2008; Okoye and Ugwu 2010). Okoye and Ugwu 2010) also reported high levels cadmium in soils from Enugu State which they claimed could serve as a source of heavy metals in animals grazing in such areas of the State.

Table 2 shows that out of 160 samples of each organ sample, 2 (1.25%) of kidney, 12(7.5%) of the liver and 44(27.5%) of the muscle samples were above the MPL of 0.5 mg/kg, 0.5 mg/kg and 0.1 mg/kg respectively. Mean concentrations of 0.0598 mg/kg, 0.0870 mg/kg and 0.1020 mg/kg in muscle samples in Nsukka, orba and Obollo slaughter houses respectively, the pooled mean concentrations of the lead in the muscle samples (0.0405 mg/kg) from all the locations is significantly (P=0.0036) lower than its MPL (0.1 mg/kg). The mean concentrations of 0.1676 mg/kg, 0.1235 mg/kg and 0.1955 mg/kg were recorded for liver and mean concentrations 0.0758 mg/kg, 0.1050 mg/kg and 0.0985 mg/kg were recorded for kidney samples in Nsukka, Orba and Obollo slaughter houses respectively. Their mean concentrations were significantly (P<0.0001) lower than their MPLs in all the locations. The higher levels of Pb recorded in organs in the present study is similar to what was reported by Bala et al. (2012a) in pigs slaughtered at Nasarawa State. The number of positive samples (1.25% and 7.5%) out of 160 samples of each organ, with concentrations higher than their respective MPL as recommended by the EC (2011). for kidney and liver (0.5 mg/kg) are small and the mean concentrations of the two organs in the different locations are significantly lower than their respective MPLs. The number of muscle samples with concentrations higher than its MPL is high at 27.5% but the mean concentration for Nsukka, Orba and Obollo slaughter houses are significantly lower than the MPL. There is no significant difference in mean concentration of lead in liver, kidney and muscle samples from Nsukka, Orba and Obollo and its MPL; again, this higher positive samples recorded in the muscle could be due to the processing method recorded in this study of the use of petrol, rubber and plastic to singe the pigs as shown in the pictures. The results from this study implies that Pb accumulates more in liver compared to other organs and is in agreement with some other studies (Iwegbue, 2008; Hiba, 2011; Bala et al., 2012a, b).

The mean lead concentrations in age range of slaughter pigs, 0 to 1 year were 0.1621 mg/kg, 0.3386 mg/kg, and 0.1467 mg/kg in kidney, liver and muscle samples respectively (**Table 3**). In age range 2 to 3 years, the lead concentrations of 0.0973 mg/kg; 0.1346 mg/kg and 0.0619 mg/kg were recorded in kidney, liver and muscle respectively. The mean lead concentrations in age range 4 to 5 years were 0.0222 mg/kg, 0.0472 mg/kg and 0.0458 mg/kg in kidney, liver and muscle respectively. The mean lead concentrations for the age range ≥6 years were 0.0467 mg/kg, 0.0946 mg/kg and 0.0375 mg/kg in kidney, liver and muscle respectively. However, mean values for lead in all the organs and muscle samples were below the MPL except in the muscle of age range 0 to 1 year (0.1467 mg/kg) which was slightly higher but not statistically significant (P=0.985). The result shows that the mean concentrations of lead decreased as the age increased, mean lead concentrations in age range 0 to 1 in kidney (0.1621 mg/kg), liver (0.3386 mg/kg) and muscle (0.1467 mg/kg) was significantly higher than the age range 2 years and above. The reason for the variation in age is because younger pigs have immature metabolic rate, innate curiosity and active calcium absorption mechanism (Hiba, 2011; Bala et al., 2012a, b; Kalu et al., 2014; Felix et al., 2016).

Generally, mean values for lead in all the organs and muscles of pigs slaughtered in the study area were below the European commission and WHO recommended maximum permissible level except in the muscle of age range 0 to 1 year, which had higher mean concentration than its MPL. This may pose serious public health threat to consumers considering the high rate of exposure recorded in this study. Consumption of lead has also been reported to be toxic in nature and even at relatively low concentrations can cause public health hazard (Santhi et al., 2008; Bala et al., 2012a).

Figure 5a-c show different ways slaughter pigs are processed in the slaughter houses. In **Figure 5a**, old tyre is used to light fire for singeing the pigs. **Figure 5b** is showing the direct use of petrol on the skin of the slaughtered pigs for faster burning and singeing at Orba and Obollo slaughter houses and **Figure 5c** is showing the use of old plastic bottle to aid the fire for singeing at Orba slaughter house. This wrong processing and singeing methods used by the butchers made the prevalence rate of the muscle higher than expected. This was in agreement with the work of Felix et al. (2016).

CONCLUSION

The pigs are exposed to lead either through feed, water, or inhalation of exhaust fume from automobile during scavenging. The potential risk posed by Pb bioaccumulation and toxicity may increase unless adequate environmental control measures are put in place. Therefore, to protect public health and ensure food safety; it is recommended that MPL for heavy metals in foods of animal origin be established in Nigeria. Measures should also be put in place by government to monitor the prevalence of heavy metals in food animals slaughtered for consumption in the studied area.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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