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Survey of Heavy Metals Residues in muscle and internal organs of Slaughtered Cattle in Enugu State, Nigeria

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Abstract
Background: *Beef is a major source of protein to man and widely consume in Enugu State. Environmental pollution and contamination with heavy metals is considered as one of the major public health problems as the metals cannot be degraded and may stay permanently in the environment The present study was undertaken to ascertain the presence and concentration of lead, cadmium and copper in muscles and organs of slaughtered cattle at Enugu State.*
Materials and Methods*: A total of one hundred and sixty (160) slaughtered cattle of liver, kidney and muscle samples of each cattle were collected and processed for the detection of lead, cadmium and copper residues using Atomic Absorption Spectrophotometer. The data generated from the study were statistically analyzed using Analysis of variance and post hoc test (SPSS version 20) and were presented in tables and percentages.*
Results*: A prevalence rate of 75 %, 70% and 78.75 % were recorded in lead, cadmium and copper in Enugu Slaughterhouse respectively. While Nsukka slaughterhouse recorded a prevalence rate of 78.75 %, 77.5 % and 82.5 % were recorded in lead, cadmium and copper respectively. Enugu and Nsukka slaughterhouses recorded a higher concentration in liver samples in lead (0.0726 ± 0.0102 mg /kg and 0.0636 ± 0.0093 mg/kg) and copper (0.0875 ± 0.0140 mg/kg and 0.0994 ± 0.0159 mg/kg) respectively. Cadmium recorded a higher level of concentration in kidney samples in both Enugu and Nsukka slaughterhouse (0.0435 ± 0.0076 mg/kg and 0.0264 ± 0.0045 mg/kg). In conclusion, the results showed that heavy metals bioaccumulate in different concentrations in slaughtered cattle in the study area. There was a statistically significant difference ($p<0.05$) in the mean concentrations of the organs tested.*
Conclusion: *However, the concentration of lead, cadmium and copper recorded in liver, kidney and the majority of muscles of the slaughtered cattle from slaughterhouses in Nsukka and Enugu fall below the maximum permissible level recommended by WHO and European commission. The levels of lead, cadmium and copper in few samples that exceeded the maximum permissible levels may pose human health threat to beef consumers in the study area.*
Key words: Heavy metals, lead, cadmium and copper

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I. Introduction

Meat and meat products form an important part of human diet. In many African countries, like in Nigeria, internal organs (liver, kidneys, heart, and lungs) are sold and consumed as a cherished food source [1]. The risk of heavy metal contamination in meat is of great concern for both food safety and human health because of the toxic nature of these substances at relatively minute concentrations[1]. Heavy metals such as lead, cadmium, copper, are naturally occurring elements in the earth's crust, and thus direct or indirect exposure to them from natural sources is inevitable especially for animals that are not intensively reared. Animals reared on contaminated pasture or fed with contaminated feed become a good source of heavy metal residues in edible animal products. Indiscriminate dumping of waste materials on land and water bodies, illegal mining of ores, painting of animals' houses, and methods of processing slaughtered cattle have been incriminated in habitual contamination of animals feeds and their products with heavy metals[2,3]. Heavy metals have been detected in muscle, liver and kidney of cattle[4, 5, 6, 7]. High levels of some toxic and trace metals in calves from a polluted area of Northern Spain was reported[8]. Lawal has reported high levels of cadmium, and lead in milk from cows grazed in open fields in Nigeria[9].

Symptoms of heavy metals intoxication include hepato-toxic effects and increase in liver function test parameters as in lead, while various cardiovascular disorders such as hypertension and cardio-myopathy, metal

fume fever, osteoporosis, reproductive impairment have been reported[1, 10]. A high dose of copper causes oxidative damage in liver in forms of granular degeneration and necrosis of hepatocytes [10, 11]. There are however very few literature of the levels of these heavy metals in muscles and internal organs in animals reared in the Enugu State environment. It is therefore imperative that this study be carried out with the major aim to investigate the possible presence and prevalence of these metal residues in organs and muscles of slaughtered cattle in the study area and also determine its concentrations.

II. Materials And Methods

Study Area

The study areas are Enugu metropolis and Nsukka urban in Enugu State. Enugu State shares boundaries with Anambra State on the West, Abia State on the South, Kogi State on the North while Benue and Ebonyi States were on the East.

The State is made up of 17 Local Government Areas, which are grouped into three Senatorial zones namely Enugu North, Enugu West and Enugu East. The population of the State is estimated by the National population Commission in 2006 to be 3,257,293.

Enugu and Nsukka are the major towns in the state. Nsukka lies at the latitude of 6°51′24″N and longitude of 7°23′45″E with a population of 309, 633 people while Enugu is on latitude of 6°27'30.12"N and a longitude of 7°30'37"E with a population of 722,664 people[12].

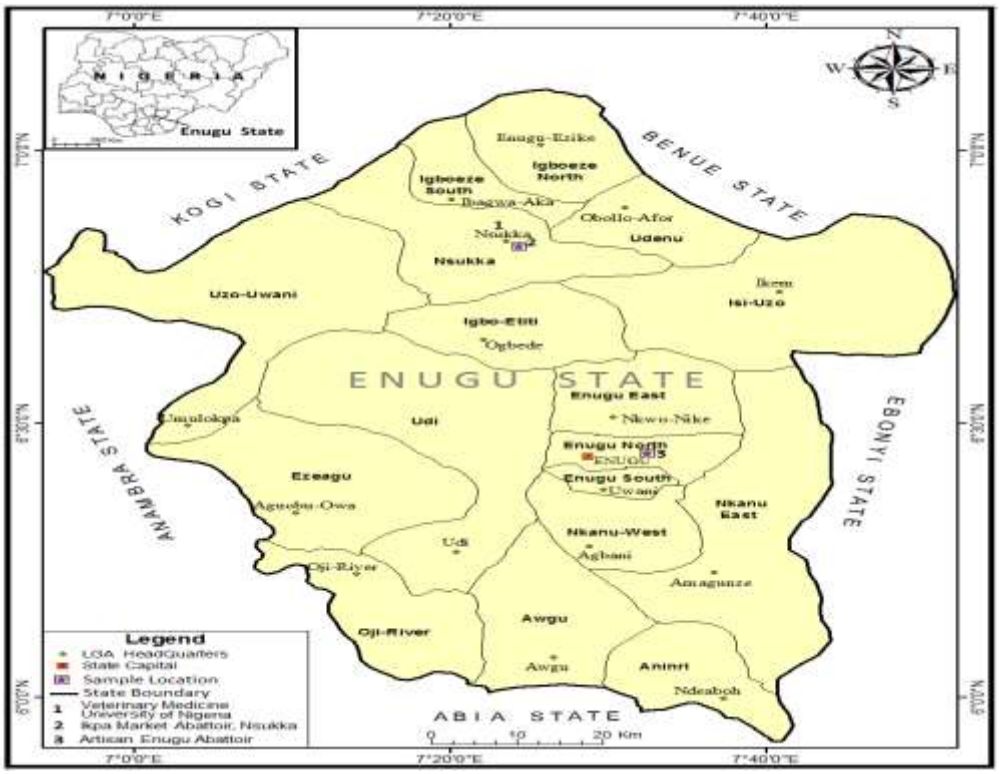

Figure 1: map of the study area showing the sample colleection pionts

Study Design

The research work was a 12 months cross sectional survey and laboratory analysis of post slaughter matrix sample from slaughtered cattle, to determine the presence and concentration of lead, cadmium and copper.

Sampling technique and Sample collection

Enugu and Nsukka were randomly selected out of the three agricultural zones in Enugu State. The two major slaughterhouses were purposively selected based on their slaughter capacity per day. Samples were randomly collected from liver, kidney and muscle of slaughtered cattle in the slaughterhouses. About 20g portion of the liver, kidney and muscle were cut from each selected cattle. Systematic random sampling was used to select one out of every three slaughtered cattle at the selected slaughterhouses, twice in a week for 12 months.

A total of 480 fresh samples of internal organs and muscle from 160 slaughtered cattle were collected between the months July, 2020 to June, 2021. The samples collected were put in different cellophane bags, labeled accordingly and tied tightly. The cellophane bags containing the samples were placed in a cooler containing ice blocks and transported to the Veterinary public health, UNN and Springboard laboratories, Awka for analysis.

Digestion of sample (wet digestion)

For the digestion of sample, 2g of the dried sample were weighed into a digestion flask after which 20ml of the acid mixture (650ml concentration (conc.) HNO_3; 80ml perchloric acid; 20ml conc. H_2SO_4) was added. Heat was applied to the flask until a clear digest is obtained. The digest was diluted with distilled water to the 100ml mark. The sample was thoroughly mixed by shaking, and 100ml of it was transferred into a glass beaker of 250ml volume, to which 5ml of conc. nitric acid was added and was heated to boil till the volume was reduced to about 15-20ml, by adding conc. nitric acid in increments of 5ml till all the residue were completely dissolved. The mixture was cooled, transferred and made up to 100ml using metal free distilled water. The sample was aspirated into the oxidising air-acetylene flame. While the sensitivity for 1% absorption was observed in the process.

Analysis

The digested cattle meat samples were examined for lead (Pb), cadmium (cd) and copper (Cu) residues under specified condition using Atomic Absorption Spectrometer (AAS) according to the manufacturer[13].

Data Analysis and Presentation

The data generated from the study were statistically analyzed using SPSS version 20.

Descriptive statistics was used to analyze the data generated which was converted to percentages, and presented in tables and graph. Analysis of variance and post hoc test was performed to determine if there was statistical significance difference in the mean concentration of lead, cadmium and copper. $P < 0.05$ was considered to be significant.

III. Results

In table 1, Enugu slaughterhouse had a prevalence rate of 72.5 %, 61.25 % and37.5 % in liver, kidney and muscle respectively in lead samples surveyed in the study area, while cadmium recorded a prevalence rate of 60 %, 63.75 % and 37.5 % in liver, kidney and muscle respectively. Also, copper was found to have 68.75 %, 72.5 % and 58.75 % in liver, kidney and muscle samples in the study area. A total prevalence of 75 %, 70% and 78.75 % were recorded in lead, cadmium and copper respectively.

In table 2, Nsukka slaughterhouse recorded 73.75 %, 71. 25 % and 56.25 % prevalence in liver, kidney and muscle respectively in lead samples surveyed in the study area, while cadmium recorded a prevalence rate of 58.75 %, 62.5 % and 40 % in liver, kidney and muscle respectively. Also, copper was found to have 80 %, 75 % and 57.5 % in liver, kidney and muscle samples in the study area. A total prevalence of 78.75 %, 77.5 % and 82.5 % were recorded in lead, cadmium and copper respectively.

Table 1: Prevalence of Lead, Cadmium and Copper in Slaughtered cattle in Enugu slaughterhouse

Heavy Metals	Status	No of Cattle (%)	No of Organ Types (%)			Total
			Liver	Kidney	Muscle	
Lead	Positive	60 (75)	58 (72.5)	49 (61.25)	30 (37.5)	137 (**57.1**)
	Negative	20 (25)	22 (27.5)	31 (38.75)	50 (62.5)	103 (**42.9**)
Cadmium	Positive	56 (70)	48 (60)	51 (63.75)	30 (37.5)	129 (**53.75**)
	Negative	24 (30)	32 (40)	29 (36.25)	50 (62.5)	111 (**46.25**)
Copper	Positive	63 (78.75)	55 (68.75)	58 (72.5)	47 (58.75)	160 (**66.7**)
	Negative	17 (21.25)	25 (31.25)	22 (27.5)	33 (41.25)	111 (**33.3**)
Total		**240**	**240**	**240**	**240**	**720**

Table 2: Prevalence of Lead, Cadmium and Copper in Slaughtered cattle in Nsukka slaughterhouse in Enugu State, Nigeria

Heavy Metals	Status	No of Cattle (%)	No of Organ Types (%)			Total
			Liver	Kidney	Muscle	
Lead	Positive	63 (78.75)	59 (73.75)	57 (71.25)	45 (56.25)	161 (6**7.1**)
	Negative	17 (21.25)	21 (26.25)	23 (28.75)	35 (43.75)	79 (**32.9**)
Cadmium	Positive	62 (77.5)	47 (58.75)	50 (62.5)	32 (40)	129 (**53.75**)
	Negative	18 (22.5)	33 (41.25)	30 (37.5)	48 (60)	111 (**46.25**)
Copper	Positive	66 (82.5)	64 (80)	60 (75)	46 (57.5)	170 (**70.8**)

	Negative	14 (17.5)	16 (20)	20 (25)	34 (42.5)	70 (**29.2**)
Total		**240**	**240**	**240**	**240**	**720**

Level/Concentrations of Lead, Cadmium and Copper in Organs samples of Slaughtered Cattle in Enugu and Nsukka Slaughterhouses

Enugu slaughterhouse had lead concentrations of 0.0726 ± 0.0102 mg /kg, 0.0465 ± 0.0071 mg/kg and 0.0146 ± 0.0031 mg/kg in liver, kidney and muscle respectively (figure 2). In Nsukka slaughterhouse lead concentrations of 0.0636 ± 0.0093 mg/kg, 0.0520 ± 0.0089 mg/kg and 0.0141 ± 0.0020 mg/kg were recorded in liver, kidney and muscle respectively (figure 3).

For cadmium, 0.0274 ± 0.0074 mg/kg, 0.0435 ± 0.0076 mg/kg and 0.0142 ± 0.0033 mg/kg were recorded in Enugu slaughterhouse for liver, kidney and muscle respectively (figure 2). While in Nsukka, concentrations of 0.0252 ± 0.0080 mg/kg, 0.0264 ± 0.0045 mg/kg and 0.0082 ± 0.0017 mg/kg were recorded in liver, kidney and muscle respectively (figure 3)

The concentrations of copper recorded were 0.0875 ± 0.0140 mg/kg, 0.0553 ± 0.083 mg/kg and 0.0267± 0.0041 mg/kg in Enugu slaughterhouse for liver, kidney and muscle respectively (figure 2). While in Nsukka, concentrations of 0.0994 ± 0.0159 mg/kg, 0.0496 ± 0.0059 mg/kg and 0.0209 ± 0.0035 mg/kg were recorded in liver, kidney and muscle respectively (figure 3).

A one-way ANOVA which was performed on data collected from Enugu slaughterhouse revealed that there was a statistically significant difference ($p<0.05$) in mean level of muscle sample when compared with other organs in cadmium. Lead and copper samples showed that there was a statistically significance different in all the mean concentrations of the organs tested. The results showed the lead had the following ($F(2,237) = (15.546)$, $p = 0.000$), cadmium ($F(2,237) = (5.293)$, $p = 0.006$) and copper ($F(2,237) = (9.898)$, $p = 0.000$).

A one-way ANOVA which was performed on data collected from Nsukka slaughterhouse revealed that there was a statistically significant difference ($p<0.05$) in mean level of the entire muscle samples when compared with other organs in lead, cadmium and copper respectively. Copper samples showed that there was a statistically significance different ($p<0.05$) in all the mean concentrations of the organs tested. The results showed the lead had the following ($F(2,237) = (11.874)$, $p = 0.000$), cadmium ($F(2,237) = (3.598)$, $p = 0.029$) and copper ($F(2,237) = (15.747)$, $p = 0.000$).

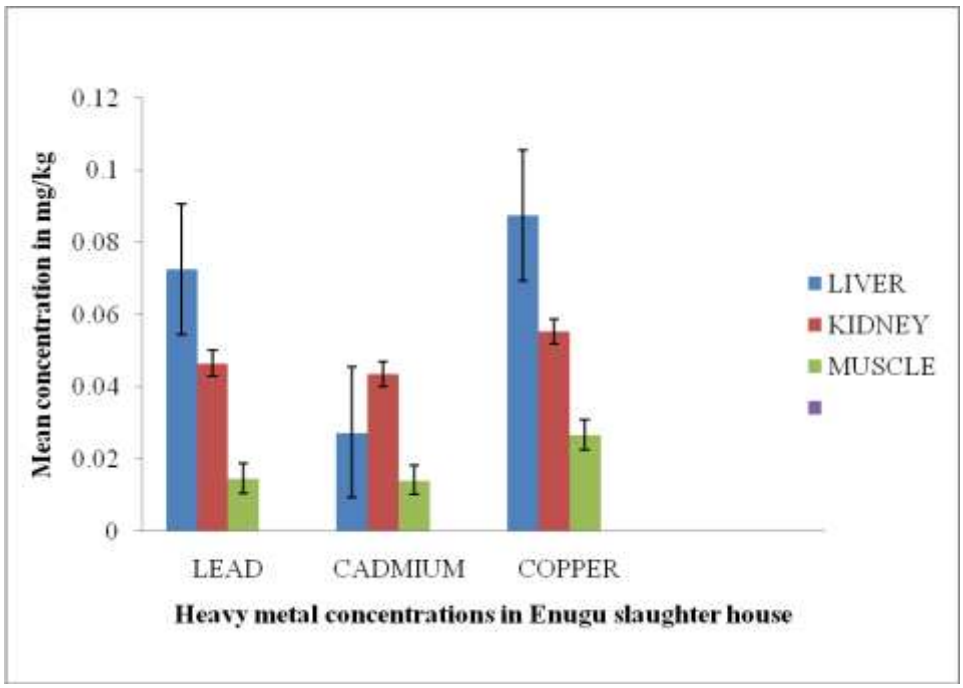

Figure 2: level of heavy metal concentrations in liver, kidney and muscle of slaughtered cattle in Enugu slaughterhouse, Enugu State

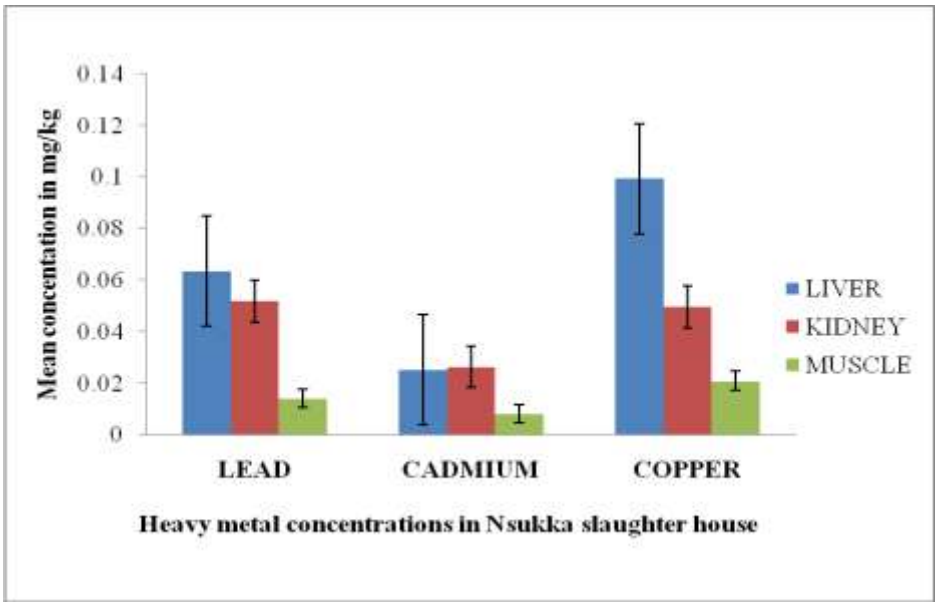

Figure 3: level of heavy metal concentrations in liver, kidney and muscle of slaughter cattle in Nsukka slaughterhouse, Enugu State

The number (%) of Organs of Slaughter Cattle with Mean Concentrations of Heavy metal above Maximum permissible Limit (MPL) in Enugu slaughter houses

For lead in Enugu slaughter house, 100% of the samples analysed were below MPL in liver and kidney. While in the muscle 96.25% of the samples fall below MPL and 3.75% of the samples were above the MPL (table 3).

For cadmium 98.75% of the samples analysed were below MPL and 1.25% were above MPL in the liver. Kidney had 100% of the samples that falls below the MPL. While in the muscle 93.75% of the samples fall below MPL and 6.25% of the samples were above the MPL (table 3).

For copper 100% of the samples in the liver, kidney and muscle falls below the MPL (table 3).

Table 3: The number (%) of Organs of Slaughter Cattle with Mean Concentrations of Heavy metal above Maximum permissible Limit (MPL) in Enugu slaughter houses

Heavy metal	Types of sample examined	No of samples examined	Maximum permissible level No (%) below	No (%) above
Lead	liver	80	80 (100)	0 (0)
	Kidney	80	80 (100)	0 (0)
	Muscle	80	77 (96.25)	3 (3.75)
Cadmium	liver	80	79 (98.75)	1 (1.25)
	Kidney	80	80 (100)	0 (0)
	Muscle	80	75 (93.75)	5 (6.25)
Copper	liver	80	80 (100)	0 (0)
	Kidney	80	80 (100)	0 (0)
	Muscle	80	80 (100)	0 (0)

The number (%) of Organs of Slaughter Cattle with Mean Concentrations of Heavy metal above Maximum permissible Limit (MPL) in Nsukka slaughter houses

For lead in Nsukka slaughter house, 98.75% of the samples analysed were below MPL and 1.25% were above the MPL in liver. While in the kidney and muscle 100% of the samples falls below MPL (table 4).

For cadmium 98.75% of the samples analysed were below MPL and 1.25% were above MPL in the liver. Kidney had 100% of the samples that falls below the MPL. While in the muscle 96.25% of the samples fall below MPL and 3.75% of the samples were above the MPL (table 4).

For copper 100% of the samples in the liver, kidney and muscle falls below the MPL (table 4).

Table 4: The number (%) of Organs of Slaughter Cattle with Mean Concentrations of Heavy metal above Maximum permissible Limit (MPL) in Nsukka slaughter houses

Heavy metal	Types of sample examined	No of samples examined	Maximum permissible level No (%) below	No (%) above
Lead	liver	80	79 (98.75)	1 (1.25)
	Kidney	80	80 (100)	0 (0)
	Muscle	80	80 (100)	0 (0)
Cadmium	liver	80	79 (98.75)	1 (1.25)
	Kidney	80	80 (100)	0 (0)
	Muscle	80	77 (96.25)	3 (3.75)
Copper	liver	80	80 (100)	0 (0)
	Kidney	80	80 (100)	0 (0)
	Muscle	80	80 (100)	0 (0)

IV. Discussion

In this study, the overall prevalence of heavy metals in Enugu slaughter house were 75 %, 70% and 78.75 % in lead, cadmium and copper of organs sampled respectively. While Nsukka recorded a total prevalence of 78.75 %, 77.5 % and 82.5 % in lead, cadmium and copper of the organs sampled respectively. The above results showed that lead samples had a higher level of heavy metal concentrations in liver samples in both Enugu and Nsukka slaughter houses (0.0726 ± 0.0102 mg/kg and 0.0636 ± 0.0093) than other organ and tissue samples. In cadmium samples, higher level of heavy metals were recorded in the kidney sample in both Enugu and Nsukka slaughterhouses (0.0264 ± 0.0045 and 0.0435± 0.0076) than other organ and tissue samples. A one-way ANOVA which was performed on data collected from Enugu slaughterhouse revealed that there was a statistically significant difference ($p<0.05$) in mean level of muscle sample when compared with other organs cadmium. Lead and copper samples showed that there was a statistically significance different ($p<0.05$) in all the mean concentrations of the organs tested. The results showed the lead had the following ($F(2,237) = (15.546)$, $p = 0.000$), cadmium ($F(2,237) = (5.293)$, $p = 0.006$) and copper ($F(2,237) = (9.898)$, $p = 0.000$). A one-way ANOVA which was performed on data collected from Nsukka slaughterhouse revealed that there was a statistically significant difference in mean level of the entire muscle samples when compared with other organs in lead, cadmium and copper respectively. Copper samples showed that there was a statistically significance different in all the mean concentrations of the organs tested. The results showed the lead had the following ($F(2,237) = (11.874)$, $p = 0.000$), cadmium ($F(2,237) = (3.598)$, $p = 0.029$) and copper ($F(2,237) = (15.747)$, $p = 0.000$). The concentration of heavy metals in the raw meat samples in the present study may be attributed to accidental or unintentional exposure to heavy metals and PAHs during grazing of the animals or industrial exposure due to air pollution[14]. The present study showed that cadmium accumulated more in kidney and liver than muscles samples. This is because kidney and liver are organs of biotransformation and detoxification. These results were similar to those reported by other researchers[15, 16, 17, 18]. Many studies conducted in Mosul City iraq revealed that environmental pollution with lead metal was higher than that for cadmium[19, 20]. From the present result, the concentrations of lead in both tissue samples were low when compared with that report of Nwude in South Eastern Nigeria[21]. The observed difference could be as a result of low industrial activities in Enugu and Nsukka when compared to Anambra State which may lead to higher contamination of environment with industrial wastes that may contain lead (Pb), cadmium (cd) and copper (Cu) materials.

V. Conclusion

From the study, it can be concluded that heavy metals bioaccumulated in different concentrations in liver, kidney and muscle in the study area. The content of lead, cadmium and copper recorded in slaughtered cattle from Enugu and Nsukka slaughterhouses in the study area falls within similar range recorded in other studies. The levels of those samples that far exceeded the maximum permissible levels may therefore pose human health threat to cattle consuming populace of the University, Nsukka and Enugu communities. It could also be concluded that the presence of the lead, cadmium and copper concentration in slaughtered cattle in the present study may be as a result of the method of rearing, environmental factors and singeing methods.

Conflict of interest

The authors have no conflict of interest.

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