

Full Length Research Paper

Determination of the presence and concentration of heavy metal in cattle hides singed in Nsukka abattoir

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Due to the health related problems associated with eating red meat, cattle hides also known as *ponmo* or *kanda*, have become a substitute for red meat in Nigeria which in turn have resulted in the increase demand for this product. To meet this demand, abattoir workers do not take time in processing these hides appropriately as different substances are used to fuel the wood to hasten the process of singeing. Unfortunately, these substance deposits some toxic metals into the hides, which is capable of causing harm to its consumers. This study aims at investigating the heavy metal contamination of the hides singed in Nsukka slaughter slab due to the various substances used to singe the hides. Samples of singed and unsinged cattle hides were collected from the abattoir and analysed for cadmium (Cd), copper (Cu), iron (Fe), nickel (Ni) and lead (Pb) using Buck Scientific Atomic Absorption Spectrophotometer (AAS). Results showed that the mean concentrations of Pb, Cd, Fe, Cu, and Ni in unsinged hides were 5.65 ± 0.70 , 1.93 ± 0.39 , 9.88 ± 1.11 , 10.45 ± 1.19 and 1.95 ± 0.12 mg/kg, respectively. In singed hides, the mean concentrations of Pb, Cd, Fe, Cu, and Ni were 4.36 ± 0.79 , 1.48 ± 0.28 , 21.60 ± 3.52 , 12.77 ± 1.06 and 2.64 ± 0.21 mg/kg, respectively. The mean concentrations of the metals with the exception of Cu in both unsinged and singed hides were above the maximum permissible level set by World Health organization (WHO) and European Commission. The results of this study proved that the environment and singeing processes may be responsible for the heavy metal contamination of hides in Nsukka abattoir.

Key words: Heavy metals concentration, singeing, cattle hides, pollution, Nsukka abattoir.

INTRODUCTION

Abattoirs are one of the industries that contribute to the problem of possible food-borne diseases and potential health hazards associated with food; especially meat

(Nemerow and Dasgupta, 1991). The slaughter of livestock to produce meat and meat products is a widespread activity and can be an important industry in

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many countries. 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 metals at relatively minute concentrations (Santhi et al., 2008).

Slaughtered ruminants such as goats, sheep and cattle are normally singed to get rid of the hair. Singeing is largely favored in many respects in African countries as it maintains the carcass hide for consumption and evokes flavors in meat that are highly acceptable by the local populace (Food and Agriculture Organization (FAO), 1985). These hides are prepared by singeing off the hair in flames fueled by substances such as wood mixed with engine oil, plastics mixed with refuse or tyres. Hides processed in this manner may contain toxic organic compounds such as polyaromatic hydrocarbons (PAH), dioxins, furans and benzene, heavy metals which can contaminate the hides (United States Environmental Protection Agency (US EPA), 1994; Agency for Toxic Substances and Disease Registry (ATSDR), 1998). Heavy metals are released during burning of hides with plastics, polystyrene polymers, tyres and woods fueled with engine oil (International Aquatic Fitness Conference (IAFC), 2000; Okiei et al., 2009). Free ranging animals as is the case in the traditional management of livestock in Nigeria can be good indicators of the general environmental pollution status (Tahvonen, 1996). Toxic trace metals can be transferred to these animals by respiration of polluted air, intake of feed or pasture contaminated with agricultural chemicals and vehicle emissions; and drinking of polluted water. These heavy metals bio-accumulate increasingly in organs and tissues of these animals (Tahvonen, 1996) and toxicity depends on dosage and length of time of exposure.

Reports have also shown that singeing of hides with hazardous substances could contaminate meat products and have adverse health implications (Okiei et al., 2009); in addition, the environment and meat processors are also at risk (Okiei et al., 2009; Holder et al., 1991). Continuous exposure and consumption of such potentially contaminated meat product poses a great source of health risk (Costa, 2000; Jayasekara et al., 1992; Leita et al., 1991). Water bodies located near the abattoir often get contaminated with these hazardous substances through bad abattoir practices, improper management and supervision of abattoir activities (Adelegan, 2002; Sangodoyin and Agbawe, 1992) including careless disposal of abattoir effluents (Sekabiru, 2010). Heavy metals such as copper, iron, chromium and nickel are essential metals since they play important roles in biological systems, whereas cadmium and lead are non essential metals but they are toxic, even in trace amounts (Fernandes et al., 2008). Heavy metals are bioaccumulative and therefore require close monitoring (Bhattacharya et al., 2008). It is therefore imperative that this study be carried out.

The aim of this study was to investigate the possible heavy metal contamination of cattle hides singed with

wood fuelled with tyres, plastics and polystyrene materials in daily abattoir operations. The research also aimed at determining the heavy metal content of waste water gotten from washing of singed hides and its role in contamination of nearby water bodies and possible effects on aquatic life.

MATERIALS AND METHODS

Study area

The study area Nsukka urban in Enugu State, Nigeria, situated at latitude 6°45' and 7°N and longitude 7°12.5' and 7°36' E (Ofomata, 1975). Nsukka is a university town with a population of 1.26 million (NPC, 2003). This abattoir is patronized on daily basis by this population and people within the environs for the purchase of meat and meat products.

Samples collection

A total number of 60 cattle (hides) were used for this experiment and were collected between the months of May to July, 2011. The veterinary approved slaughter point that was used was Nsukka Municipal Slaughter House, Nsukka, and Enugu North Senatorial Zone. Samples were collected from cattle skin immediately after flaying just before they were singed. The second sets of samples were collected after singeing of hides with wood fuelled with different substances. Ready to eat singed hides (singed – washed hides) were also used.

Processing of hides

After singeing the flayed hides, the abattoir workers soak these hides in a 208 L drum containing about 156 L of water (that is, about 3/4rd of the drum is filled with water) for about 10 to 20 min before washing them. An average of 6 hides is put into each drum. The waste water from the washing of these hides was also collected and analysed.

Sample analysis

Hides were collected directly from abattoir workers that singe them and were put in coolers containing some quantity of ice and carried to the laboratory. Each sample was scraped to remove ash and rinsed using distilled water. The samples were then drained and oven dried at 105°C for 4 h or until dried and homogenized using pestle and mortar until powdered. The powdered sample (1 g) was used for the wet digestion. This was put in a 50 ml volumetric flask. Five liters of concentrated acid mixture (HNO₃ and HClO₄) was added to the volumetric flask containing 1 g of powdered hide and shaken slightly to ensure proper mixture after which 5 ml of H₂SO₄ was also added and mixed gently. This mixture was then heated in a fume chamber for 30 min or until a clear solution was gotten. For samples in which solutions were not clear after digestion, acid solutions were added again and reheated. The flask was then left to cool and 20 ml of distilled water added to it. This mixture was shaken to ensure proper mixing. The volumetric flask was then filled to the 50 ml mark using distilled water. This was then analyzed using Atomic Absorption Spectrophotometer (AAS). The determinations were carried out in triplicates. Methods of wet digestion were adopted from Association of Official Analytical Chemist (1979) and Levinson (1968).

Statistical analyses for hide samples

The data on different variables obtained from the study were statistically analysed by using SPSS version 16 computer program. Analysis of variance and post hoc test were performed to find out the statistical differences among various parameters at $p < 0.05$. Correlation analysis was also computed to establish the relationships among various parameters (Steel et al., 1996). Ms Excel package was employed for graphical presentation of the data.

BIOASSAY

Brine shrimp lethality test (BSLT) using the singed effluents from hides to determine the LC₅₀

Water sample from washing of singed hides was collected using clean plastic containers free of debris. The water sample was analyzed with AAS. The sample was used at various dilution rates to determine the reaction of the aquatic shrimps with regards to mortality. Shrimps in distilled water were also monitored for comparison.

Determination of the concentration of the waste water effluent

The concentration of the water effluent was determined using a watch glass and a hot plate. The watch glass was weighed and recorded. The waste water (1 ml) sample was poured into the watch glass and placed on the hot plate and let to dry up. After the sample had dried to constant weight and let to cool, the weight of the watch glass was retaken and the difference between the two values was then taken and recorded as mg/ml.

Brine shrimp lethality testing (BSLT)

The method according to McLaughlin et al. (1991) was used to study the toxicity of waste water effluent. *Artemia salina* eggs obtained from a pet store in California USA were incubated in natural sea water (from the bar beach in Lagos, Nigeria) in a dam well under room condition. After 48 h, the cyst-like eggs hatched into nauplii/larvae. Ten shrimp nauplii (10) in 1 ml of autoclaved sea water were put into bijou bottles using pipette under a stereo microscope with a light source. They were separated into 4 groups of 3 each. Increasing concentration (73, 730 and 7300 ppm) of the sample was added to each group and distilled water was added to the control group. The nauplii were incubated at room temperature for 24 h after which the survivors in each group were counted.

Statistical analysis for BSLT

The results were analyzed using Finney's probit analysis (SPSS computer program) to determine the LC₅₀ at 95% confidence interval. Weak nauplii were noted as an indication of central nervous system (CNS) depression.

RESULTS AND DISCUSSION

The results from this study showed that the hides of cattle slaughtered in Nsukka abattoir accumulated varying levels of heavy metals. The mean concentrations of Cd, Cu, Fe, Ni and Pb in unsinged hides were 1.93 ± 0.39 , 10.45 ± 1.19 , 9.88 ± 1.11 , 1.95 ± 0.12 and 5.65 ± 0.70 mg/kg,

respectively.

The high concentration of heavy metals recorded in the unsinged hides may be attributed to the presence of heavy metals in the local environment which the animal could easily have come in contact with through scavenging in open waste or refuse dumps, free range grazing, drinking water from polluted streams and drains and exposure to atmospheric depositions especially from automobile fumes and open burning of solid waste (Obiri-Danso et al., 2008). These metals could also have come from various sources like vehicle emissions, tyre and engine wears, and agricultural chemicals, urban and industrial wastes (Okoye and Ugwu, 2010). Okoye and Ibeto (2008) reported high levels of lead and cadmium in soils from Enugu State which could serve as a source of heavy metals in animals grazing in such area of the state. Indeed, close correlation have been reported between heavy metals concentration in cattle tissues with that in soil, feed, and drinking water (Qiu et al., 2008).

In unsinged hides, the mean concentration of Cu and Pb were higher while Cd and Ni had lower values than those of previous studies (Table 2). Previous studies on the concentration of iron (Fe) in unsinged hides were not available when this study was carried out.

The mean concentrations of some of the metals: Pb, Cd and Cu from the present study were found to be higher than those obtained from previous works for singed treatment as shown in Table 2, while the values for Fe and Ni were lower. The differences in the concentrations of heavy metals in the various studies could be due to the different environmental factors, singeing techniques and rearing. All these values except for Cu were above the maximum permissible level set by WHO (1984) and European Commission Regulation in 2006. This report should be a source of concern on the public health implication of consumption of such hides. In singed hides, the mean concentrations of Cd, Cu, Fe, Ni and Pb were 1.48 ± 0.28 , 12.77 ± 1.06 , 21.60 ± 3.52 , and 2.64 ± 0.21 and 4.36 ± 0.79 mg/kg, respectively. Heavy metals were detected in all the samples analyzed. The mean concentrations of heavy metals determined in cattle hides are indicated as shown in Table 1. While the concentration of some metals increased, and others decreased.

Iron increased by 54.25% followed by Ni that increased by 35.38% and Cu that increased by 9.57%. Fe increased from 9.88 ± 1.11 to 21.60 ± 3.52 , Ni from 1.95 ± 0.12 to 2.64 ± 0.21 and Cu from 10.45 ± 1.19 to 12.77 ± 1.06 . The increase in the concentrations of Fe, Ni and Cu after singeing may be attributed to the fact that the hides were placed directly on metal stripes and rods or the type of substance used to wood fueled such as tyres, plastics or polyethen bags and spent engine oil, etc., as shown in Fig 1 and 2, it may also be linked to the fact the rate at which these metals are released from the substances used during the singeing process are different. The rate of intake of accumulation of these metals in the hides may

Table 1. Mean concentrations (mg/kg) of heavy metals in cattle hides in Nsukka slaughter house.

Heavy metals	USH (Mean±SEM)	SH (Mean±SEM)	SWH (Mean±SEM)	Maximum permissible level	
				EC (mg/kg)	WHO (mg/kg)
Lead	5.65±0.70 ^a	4.36±0.79 ^a	4.15±0.35 ^a	0.01	-
Cadmium	1.93±0.39 ^a	1.48±0.28 ^b	0.45±0.07 ^c	0.05	-
Iron	9.88±1.11 ^a	21.60±3.52 ^b	8.27±0.66 ^a	-	0.1*
Copper	10.45±1.19 ^a	12.77±1.06 ^a	6.08±0.56 ^b	20**	-
Nickel	1.95±0.12 ^a	2.64±0.21 ^b	2.24±0.23 ^a	0.05	-

USH: Unsinged hides, SH: singed hides, SWH: singed - washed hides, EC: European Commission, 2006; WHO: World Health Organization, 1984; * standard for Fe was set by WHO; **Values below MPL. Mean concentrations of metals within rows with different superscripts are statistically significant (post hoc test; $P < 0.05$).

Table 2. Comparison of the mean concentrations (mg/kg) of metals in the current study and previous studies.

Heavy metal	CS (Mean±SEM)	A (Mean±SEM)	B (Mean±SEM)	C (Mean±SEM)
Unsinged hide				
Lead	5.65±0.70	4.61±0.30	-	-
Cadmium	1.93±0.39	4.20±0.17*	-	-
Iron	9.88±1.11	NA	-	-
Copper	10.45±1.19	2.47±0.26	-	-
Nickel	1.95±0.12	3.50±0.17*	-	-
Singed hide				
Lead	4.36±0.79	3.06±0.26	16.35±21.28	NA
Cadmium	1.48±0.28	1.12±0.48	NA	NA
Iron	21.60±3.52	NA	46.40±10.61	206.40
Copper	12.77±1.06	5.67±1.24	11.35±3.18	NA
Nickel	2.64±0.21	2.63±0.12	NA	6.00

NA: Not Available, *Higher values in comparison to present study. Mean concentration are in mg/kg. CS: current study; A: Obiri-Danso et al. (2008); B: Okiei et al. (2009); Conc. in mg/dm³; C: Essumang et al. (2007).

hides may also be a contributing factor.

Singed treatment introduced greater concentration of Fe than any other metal by 54.25% compared to the unsinged and singed-washed. This may be attributed to the used engine oil that was constantly used to fuel the wood and/or that it occurs naturally in plants consumed by animals (Iwegbue et al., 2008). Ingestion of materials contaminated with Fe accounts for most of the toxic effects of Fe, because Fe is absorbed rapidly in the gastrointestinal tract.

It appears that the substantial heavy metal levels recorded in the unsinged hides contributed considerably to the overall values recorded with singed treatment in this study.

Lead (Pb) and Cd decrease by 22.83 and 23.32%, respectively. Singeing reduced the level of Pb by 22.83% from 5.65±0.70 in unsinged hides to 4.36±0.79 in singed hides which was not statistically significant ($P = 0.198$). The concentration of Cd decreased by 23.32% from 1.93±0.39 in unsinged hides to 1.48±0.28 in singed hides

($P = 0.321$).

The decrease in the concentration of Pb and Cd after singeing shows that heat treatment can reduce the level of these metals in hides. The fact that Pb was the least reduced of all the other metals showed that it had a slow elimination rate as reported by Humphreys (1991). Though there was a decrease in the values of Pb after singeing, the concentration gotten were still above the maximum permissible levels; therefore, this still poses a threat to human health on consumption.

The mean concentrations of heavy metals in singed washed hides as shown in Table 1 are 4.15±2.69 for lead, 0.45±0.31 for cadmium, 8.27±5.07 for iron, 6.08±4.31 for copper and 2.24±1.77 for nickel. The concentrations of all the metals reduced considerably in the singed washed hides (after proper washing).

This is indicative that proper washing during processing of hides before consumption further reduces the concentration of heavy metals in them. Although these metals reduced in concentration and were still above the

Table 3. Correlation between heavy metals in USH and SH of hides in Nsukka slaughter house.

	Fe1	Ni1	Pb1	Cd1	Cu1	Fe2	Ni2	Pb2	Cd2	Cu2
Fe1	1	-	-	-	-	-	-	-	-	-
Ni1	0.337**	1	-	-	-	-	-	-	-	-
Pb1	0.204	-0.428**	1	-	-	-	-	-	-	-
Cd1	0.027	0.543**	-0.276*	1	-	-	-	-	-	-
Cu1	0.280*	0.661**	-0.323*	0.753**	1	-	-	-	-	-
Fe2	0.474**	0.317*	-0.236	-0.245	-0.012	1	-	-	-	-
Ni2	-0.186	-0.099	-0.468**	-0.333**	-0.270*	0.299*	1	-	-	-
Pb2	0.257*	-0.322*	0.839**	-0.189	-0.180	-0.177	-0.414**	1	-	-
Cd2	-0.014	0.417**	-0.269*	0.854**	0.819**	-0.265*	-0.273*	-0.155	1	-
Cu2	-0.049	0.409**	-0.602**	0.360**	0.456**	0.220	0.335**	-0.471**	0.367**	1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

MPL except for Cu. For Pb, 100% of the hide samples were above the limit set by European Commission for Pb in hides irrespective of the treatment. This made all the 60 samples unwholesome for human consumption. Lead accumulates in the brain, liver, kidney and bones. According to Chang (1996), every 30 mg of Pb in a child's blood, causes his or her IQ to drop by 10 points. Exposure to toxic levels of Pb have also been shown to cause insomnia, nausea, headache, constipation, weight loss, anemia, malfunctioning of the kidney and reproductive organs (ATSDR, 1997b; Moore et al., 1987). This may also develop into delirium, convulsion, paralysis, coma and death (Kumar et al., 1985). Excess Pb has been shown to reduce the cognitive development and intellectual performance in children and to increase blood pressure and cardiovascular disease incidence in adults (Commission of the European Communities, 2002).

The concentration cadmium decreased steadily; the level left in the processed hide was still high. Reports have shown that Cd is toxic and unfit for human consumption and that food is one of the principal environmental sources of Cd (Baykov et al., 1996). Air borne cadmium deposits onto arable lands, where it is taken up by tobacco and food (WHO, 2010). Cd have been reported to have no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic (EU, 2002; Nolan, 2003; Young, 2005).

Iron (Fe) was detected in all the samples (100%) analyzed in each of the groups (USH, SH and SWH) and their mean concentrations were above the maximum permissible level. This is thus toxic and unfit for human consumption. Reports have shown that Fe is present in large quantity in most soil, making Fe the fourth most abundant element in the earth crust (Yahaya et al., 2009).

The concentration of Ni in singed hides was higher than that in the other groups and the values were above the maximum permissible level of Ni in food animals. Nickel is a carcinogen and therefore is of public health importance (Anonymous, 2003; Agency for Toxic Substance and Disease Registry, 2004). Small amounts of Ni are needed by the human body to produce red blood cells, but when in excess, Ni can become mildly toxic. Long term exposure to Ni can cause decreased body weight, heart and liver damage and skin irritation. Accumulation of Ni in vital body organs can cause genetic damage and cancer (Chang, 1996).

Copper was detected in all the hides analyzed, but only 20% of the hides had Cu concentrations above the maximum permissible level in both singed and unsinged hides and none (0%) of the singed-washed hides had values that were above the maximum permissible level recommended for Cu. Copper compounds are used as nutritional supplements in fertilizers and animal feed, fungicides and other agricultural chemicals and domestic sewage effluents (ATSDR, 1997). Copper toxicity is a much overlooked contributor to many health problems; including anorexia, fatigue, premenstrual syndrome, depression, anxiety, liver and kidney damage, migraine headaches, allergies, childhood hyperactivity and learning disorders.

At $p < 0.05$, correlation among elemental pairs was significant but weak. Weak correlation existed between Ni/Fe, Cd/Cu and Ni/Cu at $p < 0.01$. While at $p < 0.05$ Fe/Pb, Ni/Fe and Fe/Cu were weakly correlated.

The results showed that Pb/Pb ($r = 0.839$), Cd/Cd ($r = 0.854$), Cu/Cu ($r = 0.456$) and Fe/Fe ($r = 0.474$) pairs were positively correlated and significant at the 0.01 level in the singed and unsinged hides, while Ni/Ni ($r = -0.099$) was not statistically significant at both 0.01 and 0.05 levels as shown in Table 3.

The correlation between heavy metal pairs that were

Table 4. The mean concentration of heavy metals in waste water from washing of cattle hide.

Heavy metal	Concentration (mg/L)	WHO (mg/L)	NEMA (mg/L)
Lead	1.98	0.05	0.1
Cadmium	0.25	0.005	0.1
Iron	0.77	0.1	-
Copper	8.25	0.1	1
Nickel	1.48	0.05	1

NEMA: Maximum Permissible Limits for Discharge of Effluent or Waste Water. WHO; World Health Organization Guidelines for Drinking Water Quality (1984).

Table 5. Brine shrimp lethality test of the waste water.

Group	Concentration (ppm)	Mortality rate	
		Dead	Alive
A	7300	5	5
B	730	1	9
C	73	1	9
D	Control	0	10

negative indicates non common point source pollution, that is, pollution was probably not from the same source while those that were positively correlated may have shown common source pollution.

Water samples analysis shown in Table 4 revealed that the levels of all the metals were above the standards set by WHO and NEMA. This implies that washing of the hides after singeing may have transferred some of the heavy metals deposited on the surface of the hides as a result of singeing into the water used in washing it. It also implies that abattoir effluents discharged into nearby streams or used for irrigating nearby farm lands may be a source of heavy metal pollution in the environment.

Determination of the concentration of water effluent

Determination of the concentration of water effluent is calculated as:

Weight of watch glass (W) = 36.6452 g

Weight of watch glass + water sample (w) = 36.6525 g

$W - w = 0.0073 \text{ g} \times 1000 = 7.3 \text{ mg/ml}$

Therefore, the dry matter concentration of the water effluent was 7,300 ppm. The actual bioactive components of the waste water effluent include the already determined concentration in ppm of the elements in the hide.

The result of the BSLT in Table 5 of this study established the fact that the water effluent contains potent bioactive compounds. The compounds were rated moderate because of the slightly high LC_{50} (7316). The

ED_{50} value per general bioactivity was approximately 1/10th of the value of the LC_{50} in BSLT (McLaughlin et al., 1991). Therefore, the ED_{50} of the water effluent was approximated to be 731.6 ppm (0.732 mg/l). The surviving nauplii were dull and inactive which was an indication of central nervous system depression (Allurin et al., 2005; Mac Laughlin et al., 1991). This study showed that heavy metals concentration of water of 7.316 mg/kg may be toxic to the aquatic fauna, which by extension may affect the food chain.

Conclusion

Under the conditions of this study, it was clear that the cattle hide bio-accumulates different concentrations of heavy metals in both USH, SH and SWH. The levels of Cd, Fe, Ni and Pb far exceeded the maximum permissible levels and therefore posed human health threat to the hides consuming populace of the university and Nsukka communities. It could also be concluded that the high level of heavy metal concentrations in hide samples in the present study may be as a result of both the method of singeing, rearing and environmental factors.

The high level of heavy metals in the singed hides may also have contributed to the high concentration gotten in the hides after singeing. Therefore, investigations of heavy metals should not only be done at the abattoir, but also at farm levels, during grazing, etc., to ensure that these animals do not take in harmful quantities of metals which might show up in the processed final product. This work also revealed that waste water from hide processing in the abattoir contains heavy metals and is



Figure 1. Tyre used to fuel wood for singeing.



Figure 2. Hides placed on metal stripes during singeing.

capable of destroying the aquatic system; therefore, adequate monitoring should be done at abattoir levels to ensure that these waste are properly disposed. The potential risk of heavy metals bioaccumulation and toxicity may continue to increase in future depending on the extent of industrial influx into the environment due to man-made activities and abuses. At present, there are no set of maximum permissible levels for trace or toxic elements in hides in Nigeria; therefore, in order to protect public health and ensure food safety; it is recommended that maximum permissible levels for heavy metals in food of animal origin be established and reinforced in Nigeria.

Programs aimed at educating abattoir workers, herdsmen and other stakeholders and periodic monitoring of these metals in our environment is therefore recommended.

Conflict of interest

The authors have no conflict of interest.

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