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RESEARCH ARTICLE

ASSESSMENT OF MICROBIAL QUALITY AND HEAVY METAL LEVELS OF RAW CATTLE HIDE AND MEAT SOLD AT RETAIL OUTLETS IN TARKWA, GHANA

*Addo, M. G., Acheampong, I. A. and Akanwariwiak, W. G.

Kwame Nkrumah University of Science and Technology, Department of Theoretical and Applied Biology, Kumasi, Ghana

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ABSTRACT

A total of 384 swab samples were taken from beef, knives, wooden boards, weighing scales, shop floor as well as workers hands to assess the microbial quality of raw beef and its environmental equipment as well as the concentration of heavy metals in cattle hides available at retail outlets in the Tarkwa Municipality. Averagely 2.55±0.27 (log_{10} cfu/cm²), 2.06±0.22 (log_{10} cfu/cm²), and 1.57±0.17 (log_{10} cfu/cm²) of total viable count (TVC), total coliform count (TCC) and total Staphylococcal Counts (TSC) were recorded respectively samples from the retail outlets. There was significant microbial growth difference ($p < 0.05$) across the various retail sale environments. Microbial loads in the fresh swab sample (TVC: 1.36±0.21 (log_{10} cfu/cm²), TCC: 1.10±0.16 (log_{10} cfu/cm²), TSC: 0.87±0.13 (log_{10} cfu/cm²), were significantly lower ($p < 0.05$) than the delayed swab samples at 3.74±0.37 (log_{10} cfu/cm²), 3.02±0.30 (log_{10} cfu/cm²) and 2.28±0.24 (log_{10} cfu/cm²) respectively. Microorganisms isolated from beef and the surrounding environment included Staphylococcus spp., Salmonella, Streptococcus spp., Escherichia coli. Enterobacter spp and Klebsiella spp. The average concentrations of all heavy metal contents in hides recorded were lower than the maximum permissible limit except for Lead (Pb). The results showed that the type of processing method had a significant effect on the levels of heavy metal content recorded in hide. Although there were no significant difference ($p > 0.05$), hide processed with scrap tyre recorded the highest level of heavy metal concentration compared to fire wood-singed.

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INTRODUCTION

Even though meat has long been known for its nutritive composition, it can also serve as a rich medium of growth for harmful microorganisms. WHO (1997) reported that, meat infected with microorganisms is the cause of many food-borne diseases. Contamination with microorganisms of the quality of meat can be as results of the surroundings where these animals are kept as well as the way they are processed after slaughtering (Adeyemo, 2002). Obeng *et al.* (2013) reported in their study that, although there could be the presence of contaminant on meats, it does not necessarily constitute meat spoilage. However the presence of the microbial isolates such as *Streptococcus spp.*, S*taphylococcus spp.*, *Salmonella*, and *Escherichia coli* on meat sold in retail outlets is worrying due to their ability to cause diseases. Improper or unhygienic handling by butchers and retailers, processing, transportation, storage, sanitary conditions at various retail outlets, and environmental conditions may be the most probable sources of contamination. For highly perishable foodstuffs such as fresh red meat, the threat of food poisoning is particularly high (Nel *et al*., 2004; Yousuf *et al*., 2008). Mukhopadhyay *et al.* (2009) reported that fresh raw meat like beef have been implicated for a number of meat borne infections and intoxications in several countries. In West Africa, especially in Ghana, street-fast-processed and vended foods such as chicken and beef meat are not always well cooked and are eaten without further processing or cooking (Koffi-Nevry *et al.,* 2011). Hence, contaminated foods from fresh red meat infected with microorganisms, can lead to consumer health problems. Ologhobo *et al*. (2010) reported that microbial counts of street side roasted beef and chicken were at levels that pose health problems to consumers. Oppong-Anane and Apori (2007), indicated that meat and hides from cattle, goat and sheep were widely consumed by Ghanaians after singeing which consumers say, add on more flavour in the meat which are tolerable to the consumer and also preserve the carcass hide for consumption.

***Corresponding author: Addo, M. G.**
Kwame Nkrumah University of Science and Technology, Department of Theoretical and Applied Biology, Kumasi, Ghana.

Singeing has been an old and traditional process of burning the fur, hair or feathers superficially or slightly in order to expose the carcass of an animal. Singeing in an open fire is the major process by which hair on the skin of slaughtered cattle is removed in most African countries including Ghana (Obiri-Danso *et al.,* 2008). In Ghana, firewood is mainly used as fuel for singeing, but the relative inadequacy of firewood in current times has resulted in local butchers using scrap vehicular tyres in place of firewood (Obiri-Danso *et al.,* 2008). The use of scrap tyres, according to the local butchers is inexpensive and more efficient, as it produces more flames with less heat hence it is able to selectively burn off the fur from the animal without cracking the hide (Adam *et al*., 2013). Akwetey *et al*., (2013) reported high levels of heavy metals residues in cattle and goat hides singed with scrap tyres. Tyres Derived Fuel (TDF's) contains several heavy metals including lead (Pb), Zinc (Zn), Copper (Cu) that could be carcinogenic when exposed to consumers continuously over a long period. Unfortunately what these unsuspecting consumers probably receive is a cocktail of toxins and contaminants in meats, which may biologically accumulate in the tissues and pose health problems (Helferich and Winter, 2001). In spite of all these threats and the fact that the Environmental Health Department as well as the Veterinary Services Department of the various MMDA's in Ghana are mandated to ensure safe, healthy and hygienic meat, relatively few surveys and a lack of information on the bacteriological and heavy metal status of beef carcases offered for retail are available in Ghana. This study therefore seeks to assess the microbial quality of raw beef and its environmental equipment as well as the concentration of heavy metals in cattle hides available at the two main retail outlets in the Tarkwa Municipality of Ghana.

MATERIALS AND METHODS

Sample collection

A total of 384 replicated surface swab samples and 24 fresh hide were randomly collected aseptically during the periods from December, 2013 to January, 2014 and from March to April 2014 from two well known and most popular meat and hide selling points in the Tarkwa Municipality, Ghana. Samples were collected directly from the carcass within an hour post-slaughter and delayed market hours, in order to compare the microbial changes due to environmental temperatures and post-slaughter timings. Microbial samples collection were mainly done by surface swabs taken from 15-20cm^2 of the surface of meat-cutting equipment such as knives, wooden boards, weighing scales and carcasses meat.

Microbiological analyses

Diluted meat and equipment swabs were inoculated on nutrient agar by pour plate method for total viable count. Plates were incubated at 37^{o}C. For the isolation of Gram-negative bacteria, the identification battery included gram-staining, oxidase, citrate, urea hydrolysis, sulphide indole motility (SIM). Triple sugar iron test (TSI); briefly TSI agar was prepared in test tubes as a slant with phenol red and 1% lactose, 1% sucrose 0.1% glucose, sodium thiosulphate and ferrous sulphate were all added and incubated at 37ºC for 24 h after which it was observed for change in colour with acid/gas production. For the detection of Salmonella, one gram of each sample was also inoculated in Selenite F broth (Difco, Michigan, USA) and incubated for 18 hours at 37^{o}C. Tubes were further sub-cultured on Xylose lysine deoxycholate medium and incubated for 18 hours at 37^{o}C. Sorbitol MacConkey's agar was especially used for initial screening of E. coli. Colourless, non-sorbitol fermenting colonies were tested by serotyping. Sheep blood agar (5%), Mannitol salt agar (Merck, Darmstadt, Germany) and 6.5% NaCl Mueller Hinton agar were inoculated and incubated at 37^{o}C in a CO_2 enriched environment for the isolation and identification of Gram-positive organisms.

Processed hide collection

A total of twenty four (24) freshly singed cattle hides were obtained from the two retail outlets; Central market (Layout) and Karikwanaano, in Tarkwa for the study. Out of this number, 12 cattle hides were singed-treated with scrap tyres (T) while the remaining 12 were singed-treated using firewood (F). The control of the study was taken from the un-singed carcasses before the singing took place. The samples were labelled, packed in the ice chest containing ice packs and then transported to the Ghana Atomic Energy Commission (GAEC) Chemistry laboratory for chemical analyses.

Digestion of samples for heavy metal determination

The sample was digested and its volume was reduced to 2cm^3. The digestion was continued until the solution was colourless. This ensured the removal of all HNO_3.The sample was allowed to cool and 15 cm^3 of water was added with gentle swirling. 1M NaOH was added dropwise until a pink tinge, brown or colourless solution was produced. The solution was filtered using a Whatman filter paper No.42 followed by dilution to the mark in a 25 cm^3 volumetric flask. The digested samples were analysed for Pb, Cu, Fe and Zn concentration using SOLAAR M Atomic Absorption Spectrophotometer. All determinations were carried out in triplicate and reported as mean mineral content in mg/kg.

Statistical Analysis

SPSS Version 20 and GraphPad Prism 6 for the Graph analysis were used for the statistical analysis. A 4 X 4 factorial study design of Complete Randomized Design (CRD) was employed in this study. The probability level of significant differences (*p- value*) between sample means was set at $p < 0.05$. Concentrations of heavy metals (Pb, Cu, Fe and Zn) were expressed as mean± SEM (standard error of mean). Means of heavy metal concentrations were compared with the European Commission's Regulation Standards for Maximum Permissible Levels (MPLs) (ECR, 2006). Means of microbial contents were compared with the US Centers of Disease Control & Prevention (CDC) Standards for Maximum Microbial Permissible Levels (MPLs) (CDC, 2014).

RESULTS

Microbiological Load on Beef and Retail Environments

Meat and swab samples in this study showed high viable bacterial counts.

Table 1. Microbial contamination characteristics of meat stratified by type of sample and retailed markets (*log_{10} of cfu/cm²*)

Parameter	TVC	TCC	TSC
Type of Sample			
Fresh Swab Sample	1.36±0.21	1.10±0.16	0.87±0.13
Delayed Swab Sample	3.74±0.37	3.02±0.30	2.28±0.24
p-value	<0.0001	<0.0001	<0.0001
Effect Size (η^2)	0.4096	0.4020	0.3741
Retailed market			
Central Market	2.30±0.37	1.79±0.29	1.36±0.22
Karikwanaano	2.80±0.40	2.34±0.33	1.79±0.25
p-value	0.3687	0.2194	0.2008
Effect Size (η^2)	0.0176	0.0326	0.0353

Data is presented as means± standard deviation. p is significant at < 0.05. (η^2)-Eta square for effect size: $\eta^2 \leq 0.04$ – Weak effect, $0.04 < \eta^2 \leq 0.36$ moderate effect, $\eta^2 > 0.36$ strong effect. TVC- Total Viable Count, TCC- Total Coliform Count, TSC- Total Staphylococcal Count.

Table 2. Microbial contamination characteristics of various meat sale environments (log_{10}*of cfu/cm²*)

Parameter	TVC	TCC	TSC
Beef	5.32±0.71	4.21±0.62	3.02±0.46
Knives	2.46±0.55	1.51±0.26	0.91±0.16
Wooden Boards	2.38±0.61	2.13±0.56	1.67±0.47
Weighing Scales	1.37±0.32	1.18±0.31	0.86±0.23
Shop Floor	2.00±0.25	1.84±0.27	1.65±0.23
Workers Hands	1.78±0.25	1.51±0.17	1.34±0.37
P-value	<0.0001	0.0001	0.0006
Effect Size (η^2)	0.4787	0.4408	0.3911

Data is presented as means± standard deviation. p is significant at < 0.05. (η^2)-Eta square for effect size: $\eta^2 \leq 0.04$ – Weak effect, $0.04 < \eta^2 \leq 0.36$ moderate effect, $\eta^2 > 0.36$ strong effect. TVC- Total Viable Count, TCC- Total Coliform Count, TSC- Total Staphylococcal Count

Table 3. Microbial diversity of isolated species of swab samples from retail outlets in Tarkwa Municipality

Meat Retail Outlet	Bacteria Identified	
	Fresh Swab Sample	Delayed Swab Sample
Central Market	Staphylococcus spp.	Staphylococcus spp.
	Salmonella	Salmonella
	Enterobacter spp.	*Escherichia coli.*
		Enterobacter spp.
Karikwanaano	Staphylococcus spp.	Staphylococcus spp.
	Salmonella	Salmonella
	Streptococcus spp.	*Streptococcus spp.*
	Enterobacter	*Escherichia coli.*
		Enterobacter spp.
		Klebsiella spp.

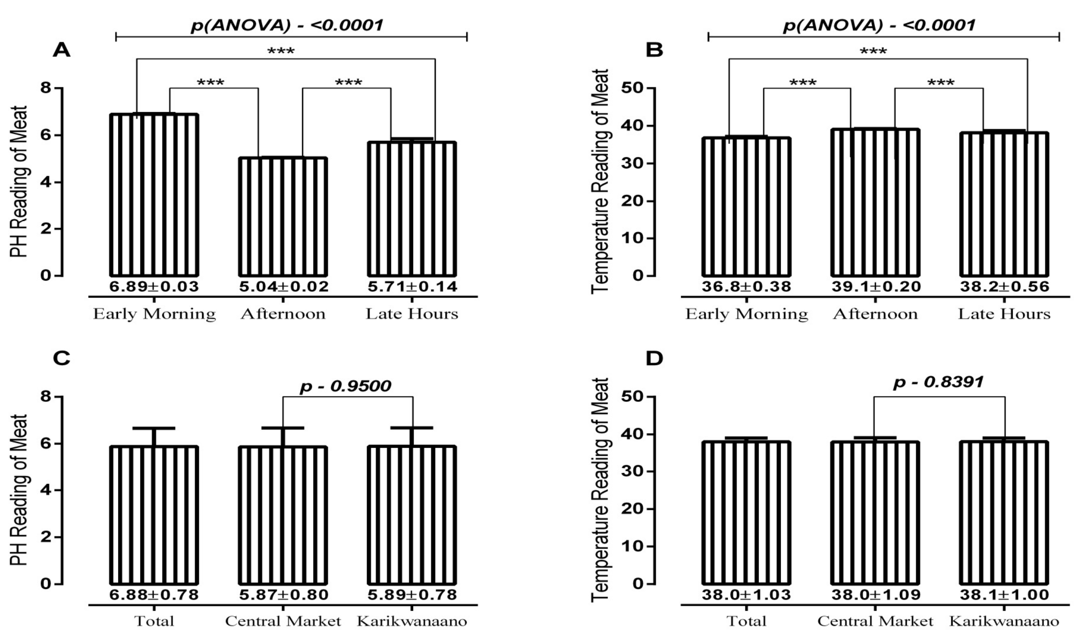

Figure 1. pH and Temperature recordings of meat stratified by time of day and retailed market

Table 4. Metal content (mg/kg) in cattle hides stratified different processing methods and retail outlet (*mg/kg*)

Parameter	Fe (Limit -50)	Pb (Limit - 0.1)	Cu (Limit – 20)	ZN (Limit – 50)
Processing Method				
Un-singed hide	9.11±4.70	0.02±0.01	10.47±2.23	15.79±1.27*
Firewood Singed Hide	22.86±5.40	0.23±0.10	20.15±4.38	26.94±2.45*
Scrap Tyre Singed Hide	38.37±5.39	0.31±0.13	27.10±4.13	67.57±0.96*
p-value	0.0250	0.6312	0.0461	0.0002
Effect Size (η^2)	0.9144	0.2642	0.8715	0.9971
Retailed Outlet				
Central Market	27.09±8.59	0.36±0.17	16.71±7.65	36.71±15.95
Karikwanaano	19.79±8.32	0.04±0.00	21.77±9.08	36.82±15.55
p-value	0.5745	0.1318	0.5014	0.9964

Data is presented as means ± standard deviation is significant at < 0.05. (η^2)-Eta square for effect size: $\eta^2 \leq 0.04$ – Weak effect, $0.04 < \eta^2 \leq 0.36$ moderate effect, $\eta^2 > 0.36$ strong effect. Fe- Iron, Pb-Lead, Cu -Copper, and Zn-Zinc. *significant different compared to permissible limit.

Microbial growth indicative of total viable count (TVC), total coliform count (TCC) and total staphylococcal count (TSC) were all significantly higher ($p<0.05$) in the delayed swab samples compared to the fresh swab samples (Table 1). Though not significantly different ($p> 0.05$) microbial loads (TVC, TCC and TSC) from the Karikwanaano retail outlets were generally higher (2.80±0.40 *cfu/cm²*, 2.34±0.33 *cfu/cm²*, 1.79±0.25 *cfu/cm²*) than that of Central Market (2.30±0.37 *cfu/cm²*, 1.79±0.29 *cfu/cm²*, 1.36±0.22 *cfu/cm²*) respectively (Table 1). It was observed that, Central market butchers and sellers primarily cleaned the meat contact surfaces/utensils twice a day. Whiles that of Karikwanaano butchers cleaned once a day. Comparatively, beef had the highest microbial loads of TVC, TCC and TSC (5.32±0.71 *cfu/cm²*, 4.21±0.62 *cfu/cm²* and 3.02±0.46 *cfu/cm²* respectively) and this could be attributed to adequate growth media conditions for microbes such as, moisture, pH as well as favourable temperature. The study recorded significant ($p<0.05$) microbial growth differences across the various retail sale environments. Comparatively, beef had the highest microbial loads of 5.32±0.71 *cfu/cm²*, 4.21±0.62 *cfu/cm²* and 3.02±0.46 *cfu/cm²* for TVC, TCC and TSC respectively (Table 2). The butchers' knives recorded the seconded highest TVC load. It was observed that the butchers' wooden boards, shop floor and hands recorded the highest TCC and TSC load in that order. Generally, the weighing scales recorded the least microbial contaminants (Table 2).

Microbiological Diversity

Different types of bacteria isolated were *Staphylococcus spp*, *Salmonella, Streptococcus spp., Klebsiella spp, Enterobacter spp and Escherichia coli* (Table 3). *Staphylococcus spp., Salmonella* and*, Enterobacter spp* were isolated from the fresh swab samples in all markets except *Streptococcus spp* that was only isolated from Layout fresh swab samples. Generally, bacteria that were isolated in the fresh swab samples were also found in the delayed swab samples at the various retail outlets. *Escherichia coli* and *Klebsiella spp* were only present in the delayed swab samples whiles *Klebsiella spp was* absent from Central market samples (Table 3).

pH and Temperature on Beef

The average pH reading of beef in this study was slightly acidic (6.88±0.78 *cfu/cm²*). The approximate neutral pH of beef in the morning (6.89±0.03 *cfu/cm²*) increased in acidity during the day peaking in the afternoon (5.04±0.02 *cfu/cm²*) before dropping during the late hours (5.71±0.14 *cfu/cm²*) (Figure 1A). From figure 1B, the temperature readings of beef were highest in the afternoon, followed by readings recorded in the late hours and lowest in the early morning. No significant difference ($p \geq 0.05$) in pH reading was recorded among the two different outlets of sales as indicated in figure 1C). No significant temperature difference was recorded among the two different outlets of sales (Figure 1D).

Heavy Metals Concentration in Hide

Comparatively, the average concentrations of all heavy metal in hides recorded were lower than the European Commission Regulations for maximum permissible levels (ECR, 2006) except for Lead (Pb) which was above the permissible limit of 0.1mg/kg (ppm). Concentrations of Zn, Pb and Cu in scrap tyre singed carcasses (67.57±0.96 *mg/kg*, 27.10±4.13 *mg/kg*, 0.31±0.13 *mg/kg* and 38.37±5.39 *mg/kg*) were all above the recommended permissible levels (Table 4). Zinc concentration was found to be highest in the hide singed with scrap tyre (67.57±0.96 *mg/kg*), but there were no significant difference in concentration between the processing methods except for Zinc (Zn) ($p<0.05$). Generally, there was a stronger effect ($\eta^2 > 0.36$) of type of processing method on the heavy metal concentration except Lead (Pb) which had a moderate effect ($0.04 < \eta^2 \leq 0.36$).

DISCUSSION

Microbiological Load on Beef and Retail Environments

The study was conducted to assess the microbial loads on raw beef and its retail equipment and the level of heavy metals (Pb, Cu, Fe and Zn) on cattle hides singed with tyres and firewood in the two main retail outlets at Tarkwa municipality in Ghana. Observations showed heavy bacteriological load of TVC, TCC and TSC carried by beef with counts ranging from 0.87±0.13 cfu/cm^2 -3.74±0.37 cfu/cm^2. Notwithstanding, the time of sampling had a strong effect on all microbial growth indicators or parameters analysed in this study. This high counts in delayed samples could be attributed to excessive handling of the meat and equipment, cross contaminations, duration of exposure as well as provision of favourable environmental conditions (optimum temperature, oxygen, moisture, nutrients) for microbial growth. Siddique *et al*., (2008), and Koffi-Nevry *et al.,* (2011), all reported higher microbial counts in late sales compared to early sales of beef offered for retail.

According to Adetunde *et al.,* (2011), microbial load of the beef builds up, as the time of exposure increases and could lead to its deterioration and unsafe for consumption. This may probably account for the increased microbial load at Karikwanaano retail outlets. Gracey *et al.,* (1999) reported that meat mostly touched by bare hands stood to be associated with remarkable changes of being inoculated with coagulase positive Staphylococci. The results from this study also agrees with the works of Soyiri (2008); Abdalla *et al.,* (2009); Obeng *et al*., 2013; Koffi-Nevry, 2011) who reported that unhygienic practices and poor handling of beef by butchers were major causes of contaminated beef. The presence of bacteria in beef and its retail equipment has widely been reported from different parts of the world (Jeffery et al., 2003; Hassan Ali *et al.,* 2010; Gurmu and Gebretinsae, 2013; Kinsella *et al*., 2008; Holds *et al.,* 2007). This could be attributed to adequate growth media conditions for microbes such as, moisture, pH as well as favourable temperature. Rao *et al.,* (2009) reported that meats have a high water content corresponding to the water activity of approximately 0.99 which is suitable for a microbial growth. The microbial loads on these equipment though insignificant ($p < 0.05$) supports the work of Ahmad *et al.,* (2013) who reported an increased in microbial loads on goat meat obtained from slaughter yards and subsequently in meat stalls at late market hours which is in-line with this current study.

Microbiological Diversity

The microbial diversity in samples from the two retail outlets is an indication of poor hygienic conditions associated in the chain of carcasses handling, processing and transportation for sale. Results indicated the predominance of Gram- negative organisms such as *Staphylococcus spp*, *Salmonella, Streptococcus spp., Klebsiella spp, Enterobacter spp and Escherichia coli.* Omorodion and Odu, (2014) and Adzitey *et al.,* (2014) all reported the presence of bacteria in meat similar to gram negative bacteria whilst Chaubey *et al.,* (2004), reported the presence of coliform bacteria in majority of raw meat and meat products.

pH and Temperature on Beef

The neutral pH of beef in the morning (6.89±0.03 *cfu/cm²*) increased in acidity during the day peaking in the afternoon (5.04±0.02 *cfu/cm²*) before dropping in the late hours (5.71±0.14 *cfu/cm²*) (Figure 1A). The physical activity of the animals for hours before slaughter, reduces glycogen concentration and plasma glucose levels below critical values and eventually leading to increased meat pH above critical range of 5.5 to 6.0 (Ndou *et al.,* 2011). Lawan *et al.,* (2011) reported that a rapid decrease of pH values at higher temperature may burst the lysosomal membrane in which some cathepsins could hydrolyze specific myofibrillar proteins. The temperature readings of beef were highest in the afternoon, followed by readings recorded in the late hours and lowest in the early morning. Our result confirms the work of Mackey and Roberts (1993) reported a range of 30°C and 39°C for the internal temperature of carcasses once the slaughtering was completed. This study had a stronger effect ($\eta^2 > 0.36$) on all microbial parameters measured (TVC, TCC and TSC). The prolong time the carcasses were openly displayed at the retail markets amidst high average ambient temperature (38.0±1.03°C) without any *in-situ* preservation could be the results of the high microbial loads on the samples.

Heavy Metals Concentration in Hide

The concentrations of all heavy metal in hides recorded were lower than the European Commission Regulations for maximum permissible levels (ECR, 2006) except for Lead (Pb) which was above the permissible limit of 0.1mg/kg (ppm). Lead is frequently the cause of accidental poisoning in domestic animals, especially cattle (Khalafalla *et al;* 2011). Generally, there was a stronger effect ($\eta^2 > 0.36$) of type of processing method on the heavy metal concentration except Lead (Pb) which had a moderate effect ($0.04 < \eta^2 \leq 0.36$). Obiri-Danso *et al.,* (2008) reported increased levels of some heavy metals, when goats and cattle hides were signed with scrap tyres. The type of material used for hide processing had significant effect on the levels of metal content recorded in hide. Hide processed with scrap tyre recorded the highest level of metal content compared with those processed with firewood.

Our results indicate that singeing of the carcasses increase the heavy metal contents in hide. This however, contradicts the work of Eremong *et al.,* (2011) and Okiel *et al.,* (2009) who reported decreasing levels of heavy metal residues in singed cattle hides. Concentrations of Zn, Pb and Cu in scrap tyre singed carcasses were all above the recommended permissible levels (Table 4). Zinc concentration was found to be highest in the hide singed with scrap tyre (67.57±0.96 *mg/kg*), but there were no significant difference in concentration between the processing methods except for Zinc (Zn) ($p<0.05$). Generally, there was a stronger effect ($\eta^2 > 0.36$) of type of processing method on the heavy metal concentration except Lead (Pb) which had a moderate effect ($0.04 < \eta^2 \leq 0.36$). According to Gautam and Irfan, (2011) when cupper (Cu) accumulates in the liver and brain in higher level of concentration it can cause Wilson's disease. Generally, the results of heavy metal residue in cattle hide was an indication that, singeing (scrap tyre or firewood) of the carcasses increased the heavy metal contents, but those signed with firewood had lower concentrations of heavy metals than scrap tyre signed hide.

Conclusion

The high microbial load on the beef and its environmental equipment surfaces in this study underscores poor level of personal hygiene and poor sanitation present right from the slaughter house to the retail outlets in the municipality. This study showed poor microbiological quality of retail beef offered for sale in the Tarkwa Municipality. The presence of *Staphylococcus spp*, *Salmonella spp*, *Streptococcus spp* and *E. coli* is worrying because of their ability to cause diseases or viable source of various diseases. A generally comparative assessment of the treatment processes showed that singeing of cattle carcasses with firewood and scrap tyres, increases the heavy metal concentrations in hides above the recommended maximum permissible limits. Environmental Inspectorate Division, Veterinary Services Directorate and all stakeholders must monitor and supervise the activities of local butchers in Ghana. This would ensure strict adherence of Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) schemes.

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