

Original Research Article

Assessment of contaminants in sacrificial meat sold at various locations in Lahore, Pakistan

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ABSTRACT

Keywords

Meat,
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Pseudomonas.

The focus of the study was to analyze the sacrificial meat sold at various sites of Lahore city for its heavy metal contents and bacterial contamination. The meat samples were collected from four different sites, Allama Iqbal Town, Istanbul Chowk, Data Darbar and Ravi Bridge. The samples were prepared by acid digestion method for heavy metal analysis. Heavy metals (Cu, Cd, Cr, Ni and Mn) are analyzed with atomic absorption spectrophotometer. Plate count method was used for the isolation of bacteria (*Salmonella*, *E.coli*, *Bacillus sp.*, *Pseudomonas sp.*, Coliform bacteria and fecal coliform bacteria) from sacrificial meat samples. Ravi Bridge site was found more contaminated as compared to other selected sites. Concentration of Cd was found higher in all samples with maximum concentration (89.1ppm) and Ni (2.12ppm) was detected only in one sample of Ravi Bridge. The statistical analysis was done to compare the heavy metal contamination in selected sites by one way ANOVA using SPSS which showed the distribution of heavy metal in all selected sites as there was no significant difference noticed. Sacrificial meat samples were more contaminated with *Salmonella sp.* and *E.coli*. There is need of proper legislation for handling of this meat as it is highly contaminated with heavy metals and microbes.

Introduction

Sacrificial meat which is also known as 'SADQA' has been used for many years in Lahore city. People give sadqa because they have a concept that by giving it we can release from all types of troubles. Many cyclists are selling sacrificial meat

in packets on road sides. The meat sellers have made it their profession and they sell one packet of meat at the cost of Rupees 5 or 10. They buy meat from Bakarmandi at the cost of 250-300 rupees. The rate of a packet fluctuates according the price of

meat in market. The meat sellers buy meat from different places for example from Bakarmandi. One packet of sacrificial meat contains 5-6 pieces of meat mainly of liver, kidney or lungs of any animal. They use dyes to color the meat. They buy meat from the shops twice a week or more than it. The stock remains with them for 3-4 days therefore they have to use dyes to keep it red. Sacrificial meat is sold at many different places in Lahore city. Allama Iqbal town, Data darbar, Ravi bridge and Istanbul chowk are the places where selling of sacrificial meat is common. The meat is highly unhygienic for the animals and birds, because heavy metals and microbes occur in this meat.

Meat is an essential part of diet of human beings and animals, also a main source for the exposure of heavy metals in the body of humans. Meat and its products are the good source of the trace elements that's why it is considered as an essential food for human being. The copper supplementation has been traced out in the meat of beef and in the muscles (Garcia-Vaquero et al. 2011). A toxic metal lead is present everywhere in nature and its toxicity has been known since prehistoric periods (Petering, 1980; Reichlmayr-Lias and Kirchgessner, 1997; Philips et al. 2011). The lead toxicity occurs largely from emissions coming from the factories and mining processes, remains of weapons stores and importantly from the making of lead batteries (Wilkinson et al. 2003). The Pb similar to Cd shows its effects regarding to the animal species, their age, their way of breeding and the form and way they enter in the organisms of body (Jarosz, 1994; Rudy, 2009).

The concentration of heavy metals in the animal meat depends on conditions of environment, genetic variations of animals

and on types of meadows. The transfer of toxic metals in humans and animals occur through food (Demirezen and Aksoy, 2004). The pollution of contamination in food sources is increasing day by day, as the rate of industrialization and urbanization is on increase. There are various types of metals in the environment that has a great danger to animals and humans (Khan et al. 1996; Sabir et al. 2003). Zn is an essential element known for the synthesis of protein, metabolism of carbohydrate, growth of cell and for the division of cell.

In Pakistan the meat is known as a by-product of many dairy and meat processing industries. Meat is a very important part of many dishes. Meat is also important as a nutritional component of food, as it provides iron, protein, zinc, energy, niacin and vitamins necessary for body organs (Romans and Ziegler, 1994). Above 370,000 animals per annum in the Pakistan are being slaughtered.

Enterobacteriaceae genus has a main pathogen containing the food products in all over the world (Alakomi and Saarela, 2009). *Salmonella* sp. of *Enterobacteriaceae* genus causes the major infection in the living organisms through the animal meat products (Plym-Forsshell and Wierup, 2006). The outbreak of salmonellosis in the living bodies caused due to the intake of contaminated meat product (Elizaquivel et al. 2009; Torlak et al. 2012).

Infected knives and different equipment are used to slaughter the animals in unclean conditions. The remains of the body organs are carried out to the different places and to the vendor shops openly in the unclean conditions. These meat organs are openly displayed in the butcher shops

or on the roadsides. No protection is used for the prevention of the meat from contamination. There are some factors like sun light, humidity, ambient temperature and some flies that cause the transfer of microbes from one place to another (Aftab et al. 2012).

Livestock provides a main source to the *Salmonella*, which stay in the intestine and expelled through the feces (Zadik et al. 1993). The slaughter houses which are working at local scale provide bacteria favorable condition to grow and contaminate the meat. The high load of microbes in the shops improves the probability of initial contamination of meat (Bhandarea et al. 2007).

Many scientists from different countries have been studied the occurrence of *Campylobacter* in the raw meat. It has been concluded that the occurrence of this bacteria is 50% or more than it in some cases (Pennacchia et al. 2009).

The current study was carried out assess the heavy metals contamination in sacrificial meat sold at various locations in Lahore city. Isolation and identification of bacterial species present in sacrificial meat was another focus of the study.

Materials and Methods

Sampling of meat

The different meat samples were collected from four various sites of Lahore. Allama Iqbal Town, Data Darbar, Ravi Bridge and Istanbul Chowk were the sites from where samples were collected.

Analysis of heavy metals

10gm of each sample were weighed on the

weighing balance. The samples were placed in the china dish and then place in the oven for drying process at 80⁰C for 48 hours. After oven drying, the samples were ground with the help of pestle and mortar.

After grinding the meat samples were sieved to get fine powder. Acid digestion technique was adopted for the analysis of heavy metals by using HNO₃. Filtered the samples with the help of filtration assembly.

Atomic absorption spectrophotometer analysis:

The analysis of heavy metals such as Cu, Cd, Cr, Mn and Ni was done with the help of Atomic Absorption Spectrophotometer (FAAS, Shimadzu AA-7000F).

Statistical analysis of heavy metals:

The comparison of heavy metals between the various sites was done using one way ANOVA in SPSS software version 19.

Isolation of microbes in the sacrificial meat

Pre- treatment

Twelve gram of meat sample was weighed from all four different samples and 100ml of Buffered Peptone Water (BPW) was added which gives 1:10 ratio of dilution for each sample. Then sample was homogenized in the orbital agitator

Culture media used

For the initial growth of the microbes a medium was used which was prepared by the nutrient agar (Prasad, 2000).

Sample dilution

1ml from the homogenized meat was added with the help of pipette into a test tube having 9ml of the solution of BPW. That was the first dilution from it second dilution was made by transferring 1ml to the second tube containing 9ml BPW with another pipette to avoid contamination. Repeated the procedure till the 10th dilution was prepared. Tubes were shaken to mix the solution well.

Pouring of agar

Took 1ml from each dilution and poured them into each petri dish. 2-3ml of Nutrient agar was poured in each petri plate. Then mix the dilution of samples uniformly so that a uniform layer will be formed. In the end allowed the medium to get solidify

Incubation of the prepared medium

After the pouring process inoculate the petri dishes with each diluting and incubate the plates for 48 hours at 32°C.

Counting of the colonies

After the incubation process counted the colonies by the colony meter in each petri plates containing almost 30-60 colonies. The result was recorded as per dilution.

Identification of *Salmonella*

12gm of the meat samples from all four samples were taken and dissolve in the 100 ml of BPW. Then the mixture was agitated for 15-20mins to mix it completely. The meat samples which were agitated and transferred to the 500ml round bottom flask in the oven for incubation at 37° C for 18-20 hours. From the sterilized bottle 10ml of meat sample

was transferred to Tetrathionate broth and same quantity of meat sample was also taken and transferred to the selective medium and incubated at 42-43°C for 48 hours. After incubating the media, the streaking was done on the *Salmonella* / *Shigella* agar on each petri plate. After streaking the plates placed them in the incubator for 24 hours at 37° C. After twenty four hours the *Salmonella* colonies were examined.

The colonies of *Salmonella sp.* appeared in pink colored colonies with pinkish to red boundaries.

Detection of *Bacillus sp.*

MacConkey agar was used for the detection of *Bacillus sp.* The preparation of agar was done by adding 5gm of agar and 100ml deionized water. The medium was then sterilized in the autoclave and shifted to the petri plates which were also sterilized. The agar plates were than cooled and dried. Placed the agar plates in the laminar air flow till medium get solidify. After medium get solidify inoculation was done by the culture of bacteria by streaking method. Then the plates were placed in the incubator for 24 hours at 37° C (Cheesebrough, 1993).

Detection of *Escherichia coli*

EMB agar was also prepared for the detection of the *Escherichia coli*. Presence of shiny reddish green colonies represented the positive result.

Detection of *Pseudomonas sp.*

For the detection of *Pseudomonas sp.* the pseudomonas agar was used. Growth indicated the presence of *Pseudomonas* colonies.

Total coliform bacteria analysis in the sacrificial meat samples

Preparation of homogenate sample

Twelve gram of meat was taken and dissolved in the 100ml of each sample. Placed each sample in the agitator for mixing the solution for 15-20 minutes.

Preparation of EC broth:

EC broth is a selective medium used for the identification and differentiation of coliform and fecal coliform bacteria. The culture media of these samples were prepared by adding 28 gram of EC broth in the 500 ml of the deionized water.

Preparation of dilutions

The dilutions for each sample were prepared by making 5 dilutions of 10ml, 5 dilutions for 1ml and another 5 dilutions of 0.1ml for each sample separately and one blank sample. Cotton plugged and covered the test tubes with aluminum foil tightly. Test tubes were sterilized at 121°C for 15 minutes in the autoclave.

Determination of Coliform bacteria

Total coliform bacteria were analyzed in the meat samples. MPN method was used to determine the number of bacteria present in each sample. Then took the homogenate mixture of meat and add them into the dilutions.

Analysis of Faecal coliform

The fecal coliform bacteria were counted with the help of MPN method in the samples of sacrificial meat. The number of fecal coliform bacteria was counted in MPN/100ml.

Results and Discussion

Contamination of Metals in Sacrificial meat

The concentration of metals in different sacrificial meat samples were analyzed by Atomic Absorption Spectrophotometer after preparing the sample with acid digestion technique. The higher concentration of Cu was obtained in the sample collected from Ravi Bridge (0.20ppm) and lowest in sample from Istanbul Chowk (0.06ppm). The concentrations in the samples from Allama iqbal town (0.176ppm) and in Data Darbar (0.07ppm) were also higher than Istanbul Chowk concentration. The allowable limit for Cu is 40mg/kg (Visnjic-Jeftic et al. 2010). The observation shows low concentration of copper in the meat samples (Figure 1). The concentration was observed in different organs of the body and which was less than the 200ppm which is the allowable limit of FAO/WHO, (2000). Figure 2 indicates the higher concentration of cadmium in the sample collected from Ravi Bridge (86.01ppm) and lowest concentration in the sample collected from Allama Iqbal Town (12.62ppm). The concentration of samples from Istanbul Chowk (77.27 ppm) and Data Darbar (66.01 ppm) were also higher than the Allama Iqbal Town. The allowable limit given for Cd in the kidney of organism is 1ppm and for the liver is 0.5ppm. In the current study the concentration observed in all sample is higher than the given limit. . Visnjic-Jeftic et al. (2010) observed the concentration of Cd in meat 0.124 µg/g.

The concentration of chromium was determined at all four sites (Figure 3). Istanbul chowk have the highest concentration of chromium (0.375ppm) than others, Allama iqbal town

(0.347ppm), Data darbar (0.298ppm) and Ravi Bridge (0.286ppm). The allowable amount of chromium is 2.3mg/kg. Visnjic-Jeftic et al. (2010) also obtained the chromium concentration in the meat of liver (1µg/g) higher than the meat of beef

(0.23µg/g). Ni concentration was higher in samples collected from Ravi Bridge (0.708ppm). However its concentration was found to be below detectable limits in all other sites (Figure 4).

Figure.1 Heavy metal concentration of Cu at various sites by acid digestion method

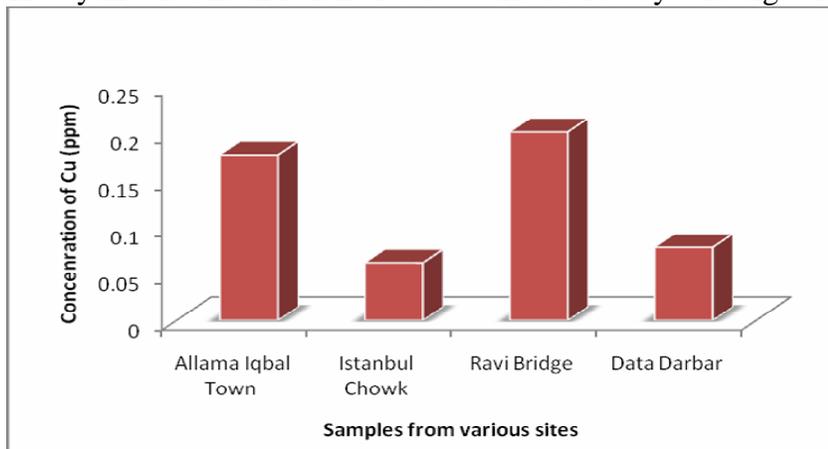


Figure.2 Heavy metal concentration of Cd at various sites by acid digestion method

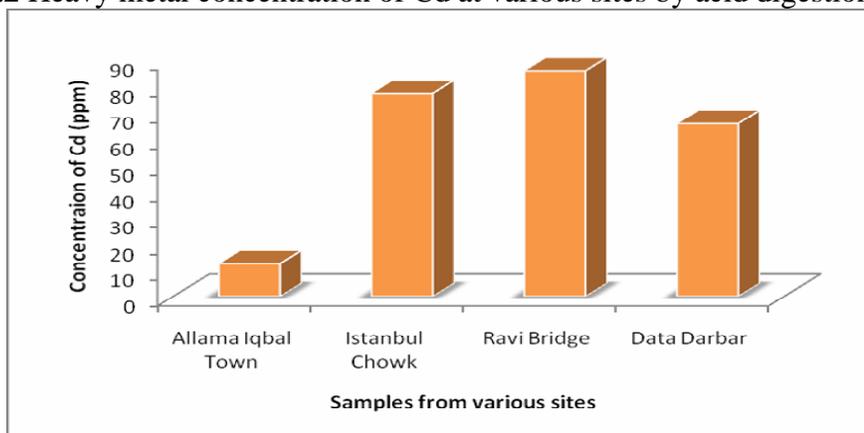


Figure.3 Heavy metal concentration of Cr at various sites by acid digestion method

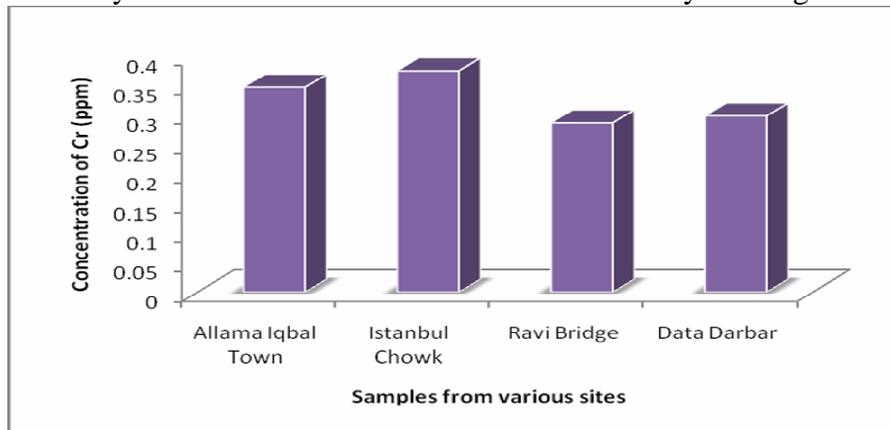


Figure.4 Heavy metal concentration of Ni at various sites by acid digestion method

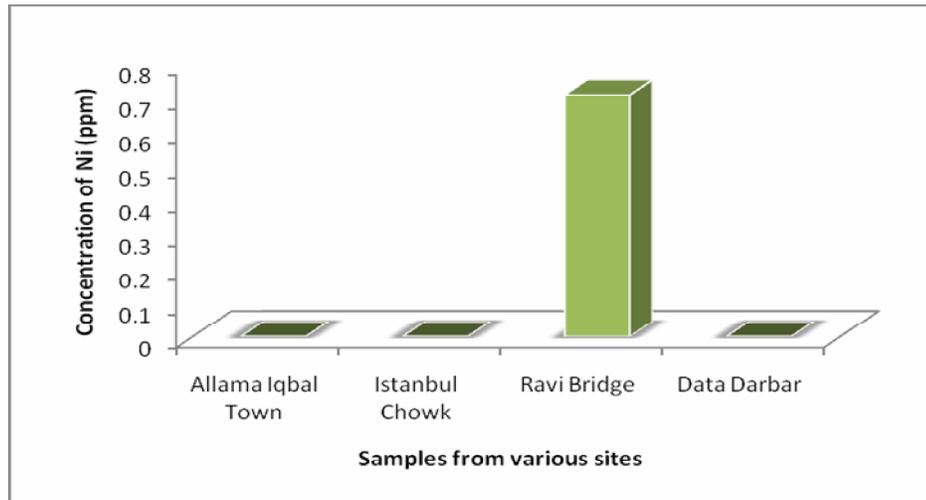


Figure.5 Heavy metal concentration of Mn at various sites by acid digestion method

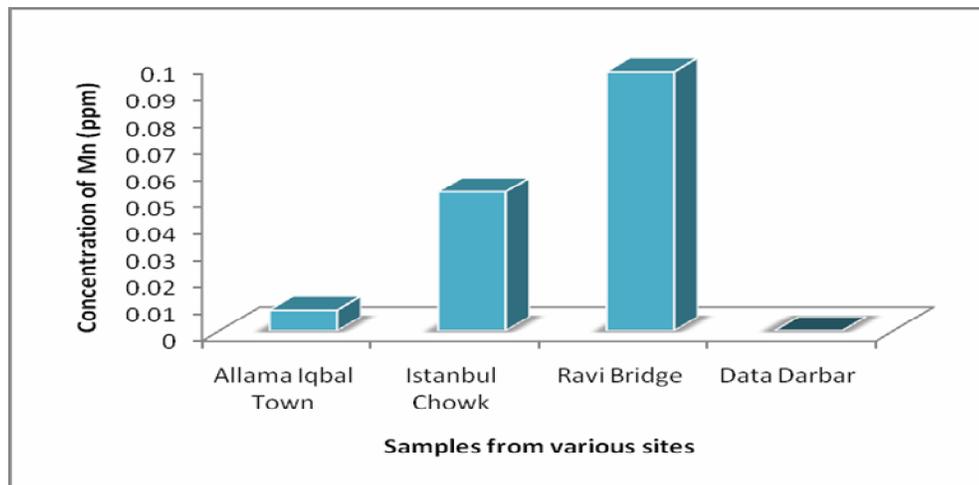


Table.1 Analysis of Coliform and fecal Coliform bacteria in the sacrificial meat samples

Type of bacteria	Ravi Bridge (MPN/kg)	Allama iqbal town (MPN/kg)	Data Darbar (MPN/kg)	Istanbul chowk (MPN/kg)
Coliform	8.3×10^4	2.4×10^4	4.8×10^4	1.1×10^5
Fecal coliform	1.4×10^2	1.0×10^2	1.1×10^2	1.1×10^2

Table.2 Isolation of bacteria from meat samples collected from four different sites on selected media

Samples	EMB agar	MacConkey agar	Pseudomonas agar	Salmonella agar	Results
Ravi	+	-	+	+	<i>E.coli</i> <i>Pseudomonas sp.</i> <i>Salmonella sp.</i>
Allama iqbal town	+	+	-	+	<i>E.coli</i> <i>Bacillus sp.</i> <i>Salmonella sp.</i>
Data darbar	-	+	+	+	<i>Bacillus sp.</i> <i>Pseudomonas sp.</i> <i>Salmonella sp.</i>
Istanbul chowk	+	-	+	+	<i>E.coli</i> <i>Salmonella sp.</i> <i>Pseudomonas sp.</i>

Table.3 One way ANOVA showing relationship of heavy metals within and between the various sites
ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Copper	Between Groups	.040	3	.013	.684	.586
	Within Groups	.157	8	.020		
	Total	.197	11			
Chromium	Between Groups	.020	3	.007	1.188	.374
	Within Groups	.044	8	.006		
	Total	.064	11			
Cadmium	Between Groups	6014.892	3	2004.964	2.362	.147
	Within Groups	6791.635	8	848.954		
	Total	12806.527	11			
Manganese	Between Groups	.014	3	.005	.508	.688
	Within Groups	.074	8	.009		
	Total	.088	11			
Nickel	Between Groups	1.124	3	.375	1.000	.441
	Within Groups	2.996	8	.375		
	Total	4.120	11			

Table.4 Comparison of metals in sacrificial meat and commercial meat

Metals	Sacrificial meat (ppm)	Commercial meat (ppm)
Cu	0.44	0.31
Cd	89.10	17.93
Cr	0.43	0.52
Ni	2.12	10.44
Mn	0.26	0.03

As the Ravi Bridge samples contain kidney and liver pieces that may be the reason for more concentration of Ni because these organs accumulate more heavy metals as compared to other organs of the body. Akan et al. (2010) studied the amount of nickel in the different organs of the body and the value observed was in between the 0.01-0.22 $\mu\text{g/g}$ and highest concentration was seen in the chicken liver however low value in the meat of caprine (0.1 $\mu\text{g/g}$). The allowable amount of chromium is 5mg/kg (FAO/WHO, 2000).

The highest concentration of Mn was seen in Ravi Bridge (0.0969ppm). However, Data Darbar showed concentration of Mn below detectable limit and other two sites Istanbul Chowk (0.0522ppm) and Allama Iqbal Town (0.0078ppm) shows concentration level near to highest concentration of Ravi Bridge (Figure 5). Visnjic-Jeftic et al. (2010) observed the manganese (4.11 $\mu\text{g/g}$) concentration higher in the chicken liver than the meat of beef (0.45 $\mu\text{g/g}$).

Bacterial contamination in sacrificial meat

Coliform and fecal coliform bacteria comparison at various sites

The value of fecal coliform bacteria MPN/kg of all samples collected from

Ravi Bridge (1.4×10^2) was highest of all other samples however the count was less than this value but close to it in other samples of Data darbar (1.1×10^2) Allama iqbal town (1.0×10^2) and Istanbul chowk (1.1×10^2) (Table 1). In the current study Faecal coliform bacteria were identified in the all sacrificial meat samples collected from different sites while in previous studies Bhandare et al. (2007) isolated these bacteria from the meat samples collected from different butcher's shops. The large number of coliform bacteria was counted in the samples collected from the Ravi Bridge (8.3×10^4) and other samples of Data darbar (4.8×10^4) and Allama iqbal town (2.4×10^4) and Istanbul chowk (1.1×10^5) showed less number of fecal coliform in the meat samples. The study done by Odumeru and Belvedere (2002) identified the higher value of coliform bacteria in the meat samples while in present study these Coliform were also identified in great number in the different samples of meat

Isolation of bacterial species in all samples collected from different sites:

In Ravi sample shiny reddish green and pink bacterial colonies were identified as *E.coli*, *Pseudomonas sp.* and *Salmonella sp.* on EMB agar, *Pseudomonas* agar and salmonella agar respectively (Table 2). Svilokos et al. (2004) identified the *Salmonella sp.* and *E.coli* together in the

meat samples. They calculated the percentage of these two bacteria *Salmonella sp.* (12.5%) and *Escherichia* (7.5%) in total samples.

In Data darbar sample three types of bacteria were identified *Bacillus sp.*, *Pseudomonas sp.*, *Salmonella sp.* showing pink opaque with pinkish to red boundaries on the MacConkey agar, pseudomonas agar and salmonella agar respectively. Dominguez et al. (2002) studied the presence of *Salmonella sp.* and *Campylobacter* together in the food samples. But the percentage of *Salmonella sp.* (17.67%) was higher than the *Campylobacter* (34.34%).

In sample collected form Allama iqbal town *E.coli*, *Bacillus sp.* and *Salmonella sp.* showing shiny reddish green, pink opaque and pinkish colonies grown on the EMB agar, MacConkey agar and Salmonella agar respectively. Audenaert et al. (2010) identified *Lactobacillus sp.* in their different meat samples found with the Lactic Acid Bacteria.

In Istanbul chowk sample three species of bacteria were identified such as *E.coli*, *Bacillus sp.* and *Salmonella sp.* on the EMB agar, MacConkey agar and Salmonella agar respectively showing the colonies of shiny reddish green and pinkish on the medium. Russo et al. (2006) isolated the different species of *Pseudomonas* in different organs of meat. Aftab et al. (2012) observed two bacteria *Salmonella* and *E. coli* but focused on the infection through *Salmonella* during the meat processing and unwashing of meat.

Statistical analysis of heavy metals in sacrificial meat:

The one way ANOVA determine the

significance value of heavy metals in the various sites. The ANOVA analysis showed that there is no significant difference of the concentration of copper (0.58), Cd (0.147), Cr is (0.374), Mn (0.688) and Ni (0.441) are all greater than 0.05.

Comparison of sacrificial meat and commercial meat contamination:

Five heavy metals were determined in the sacrificial meat and similar study was conducted on commercial meat and it was concluded that sacrificial meat was more contaminated than commercial meat (Table 4).

Sacrificial meat has been sold at various locations of the Lahore city. As meat is sold after one day and it becomes fade therefore meat vendors use dyes to give red color to the sacrificial meat. This research was conducted for the evaluation of the heavy metals and microbial contamination in the sacrificial meat samples. The sacrificial meat was fully contaminated with the microbes and heavy metal contents. The concentrations of heavy metals exceed the standard given by FAO/WHO. *Salmonella sp.*, *E.coli*, *Bacillus sp.*, *Pseudomonas sp.*, coliforms and fecal coliform bacteria were isolated in the sacrificial meat. These bacteria were present in large number in the sacrificial meat. So it is concluded that the high rate of heavy metal concentration and microbial contents in sacrificial meat can make it more hazardous and harmful to the health of animal and birds.

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