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RESEARCH ARTICLE

Comparative Study of Heavy Metals Residues and Histopathological Alterations in Large Ruminants from Selected Areas around Industrial Waste Drain

Summaira Sharaf[1], Muti-ur-Rehman Khan[1], Asim Aslam[1] and Masood Rabbani[2]

[1]Department of Pathology; [2]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: drniazi@uvas.edu.pk, summaira_sharaf@yahoo.com

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ABSTRACT

Heavy metals (Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni) residual concentration as well as histopathological changes in intestine, kidney, liver and muscles of slaughtered large ruminants i.e cows and buffaloes (n=5 from each specie) were carried out at 2 different sites near industrial zone drain. Atomic absorption spectrometry (AAS) was used to determine residual concentrations of heavy metals in organs. Results exhibited that organs were found higher in fixation of heavy metals as assimilate to the maximum permissible limits. The levels of heavy metals in the intestine, kidney, liver and muscles of large ruminants were ranged from Cr; 0.82±0.36 to 6.47±0.68, Cu;10.58±1.50 to 40.52±10.71, Zn; 19.60±0.84 to 36.72±0.75, Pb; 0.40±0.17 to 2.49±0.30, Fe; 40.22±0.29 to167.13±9.47,Mg ;123.73±4.45 to 135.74±0.93, Mn;1.54±0.30 to 8.49±0.88 and Ni; 0.73±0.49 to36.72±0.75. The concentrations of all the metals in the intestine, liver, kidney and muscles were found to be statistically significant ($P<0.05$) except few results. Hence, the concentrations of Cr and Pb were higher than 1 mg/kg permissible limit set by FAO/WHO. Prolonged and persistent use of such water for irrigation of agricultural lands and as feeding source for animals and humans might have toxic effects. Residual effects of selected organ samples of selected animals were high in heavy metal concentrations and sever histopathological alterations were also noticed in organs of selected area which were closer to industrial drainage. These selected organs were not fit for human consumption.

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INTRODUCTION

Heavy metals pollution to the natural environment is an immersing issue of prime importance. Their bioaccumulation potential and toxicity are being transferred to human beings through the food chain (Gagne *et al*., 2013; Tahir *et al*., 2017). To maintain the body metabolism, metals are essential but their higher concentration is toxic (Abii and Okorie, 2011). With food, air and drinking-water minor amount of heavy metals can enter the animal bodies however bioaccumulation of the heavy metals is poisonous. Some toxic metals are responsible for the spontaneous cell death by promoting the production of the superoxide (O^{2-}) and paramagnetic anions (Davis, 2010). The heavy metals enhance free radical formation and become toxic to the enzyme system and also compete with nutritional minerals absorption by converting into metallo-enzyme complexes (Govind and Madhuri, 2014).

In the body of living animals, these heavy metals exert toxic effects like damage to kidneys, liver, alteration in levels of different biochemical and hormones which cause severe health problems in animals and human beings inflicting huge economic losses. The pollution of heavy metals to coastal environment is under prime interest of environmental investigators (Bayen *et al*., 2005). Bioaccumulation of some heavy metal may influence the availability of some indispensable metals in the body of an organism. Contaminated location is directly reflected by metal residual distribution in ecosystem. Understanding of toxic effect is also facilitated by assessing extent of feed and water contamination, along with heavy metal toxicity and as well as its bioaccumulation and durable bio magnifications in living organisms (Has-Schon *et al*., 2006; Saleemi *et al*., 2019). Observing the status of natural water bodies through biological monitoring along with estimating the levels of heavy metals in environment by chemical analysis are good tools to check this issue (Liu *et al*., 2007).

Contaminated food and water are basic source of heavy metals in animals. These animals are being consumed as food source. This metal contaminated meat is not safe to consume and can be toxic for human health. Bio magnification is process of accumulation of toxic substances (metals etc.) into food chain. On the basis of above review, it is clear that wastewater use for irrigation is playing an important role in the bio magnification of heavy metals. There is a chain process through which these metals contaminate the entire food chain ultimately leading to the contamination of whole ecosystem. Soils are being contaminated due to wastewater use of irrigation. Vegetation receives metals from the contaminated soils and these metals then indirectly, accumulates into the animals consuming such plants.

Due to establishment of numerous industries in and around the Lahore city and non-observance of standard protocols for deposition of toxic industrial effluent, the concentration of toxic materials specially that of heavy metals is assumed to be higher than that of recommended range. Higher concentrations than permissible levels of heavy metals in organs of animals are believed to cause physiological and histopathological disorders resulting in economic losses and public health issues. Therefore, in current study, attempts were made to evaluate the residual effects of heavy metals and histopathological changes in certain organs of cows and buffaloes in selected areas of Lahore industrial zone.

MATERIALS AND METHODS

The research was conducted at Applied Chemistry Research Centre (ACRC) in Pakistan Council of Scientific & Industrial Research (PCSIR) Lahore, Pathology Laboratory in Department of Pathology and Department of Environmental Sciences in University of Veterinary and Animal Sciences (UVAS), Lahore.

Heavy metal (Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni) residues in organs of large ruminants were analyzed at two selected points i.e. Hudiara Drain. Industrial zone having different types of industries (leather, textile, bicycle manufacturer and pesticides etc.) around the selected main drain. The industrial drainage waste water of above mentioned industries was added to main drain water before the first selected point. First selected point was near to leather industries. Second point was 2 Km away from first point. To assess the effect of heavy metals (Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni) on animal health, attempts were made to collect the organs (intestine, kidneys, liver and muscles) of selected slaughtered animal species raised in selected areas 1 and 2. Residual effects of heavy metals were measured by digestion and atomic absorption spectrometry (AAS). The animals selected for study were consuming drain water directly or fodder irrigated with industrial waste. Histopathology of above mentioned organs was also performed to observe histological alterations.

Research plan / Scientific / Technical methodology: schedule / Phasing

Phase-1: Analysis of heavy metals residues in organs of large ruminants: To assess the effect of heavy metals (Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni) on animal health, attempts were made to collect the organs (intestine, kidneys, liver and muscles) of selected animal species (buffalo and cow) raised in selected areas 1 and 2. Samples(n=5) were labeled with different marking pattern and numbers including dates and shifted to Environmental Sciences Laboratory at UVAS, Lahore and PCSIR, Lahore. The samples were processed by wet digestion (Richards, 1968) and residual effects of heavy metal was assessed by atomic absorption spectrometry (AAS) as described by Licata *et al*. (2004).

Phase-2: Histopathological alterations in organs of animals raised in the vicinity of drain: For further toxico-pathological studies intestine, kidneys, liver and muscles samples of large ruminants (buffalo, cow) reared at the selected sites were collected. Samples (n=5) from each specie i.e. cattle and buffalo from two selected sites were collected randomly and processed for analysis. Histopathology of these organs was performed. 10% neutral buffered formalin was used to preserve all tissue specimens for histopathology. All the tissue samples were processed for routine H and E staining as described by Bancroft and Gamble (2008) and examined under microscope.

Statistical analysis: All the data were presented as the mean ± SD (standard deviation) and were subjected to analysis of variance (ANOVA) using SPSS version 23 software. Difference between groups regarding organ residues parameters were determined by using independent T-Test. Statements of statistical significance were based on a probability of $P<0.05$.

RESULTS

In the present study, heavy metal residual concentration of Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni (mg/L) in intestine, kidney, liver and muscles of slaughtered buffalo and cow were determined from two selected sites of study area as shown in Table 1 and 2 respectively. While histopathological lesions in stained sections of tissues of slaughtered buffalo and cow were more pronounced at site 1 of this study as shown in Figure A, B, C and D.

Residual concentration of heavy metals and histopathological alterations in different organs of cows and buffaloes

Intestine: Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni (mg/L**)** residues in intestine of slaughtered buffalo in sampling area 1 were high than area 2. Intestine from animals of site 1 showed significant difference ($P<0.05$) in heavy metal concentration (Cr, Cu, Pb, Fe, Mg, Mn and Ni) as compared to site 2 while a non-significant difference was observed regarding concentration of Zn in samples of animals of both sites as shown in Table 1. It is evident from Table 2 that concentrations (mg/L) of Cu, Zn, Pb,

Table 1: Comparative mean ± SD values of Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni (mg/L) in intestine kidney, liver and muscles of buffalo from two different sampling sites of study area

Name of metal	Intestine			Kidney			Liver			Muscles		
	Site 1	Site 2	P-Value	Site 1	Site 2	P-Value	Site 1	Site 2	P-Value	Site 1	Site 2	P-Value
Cr	6.11±0.24	5.68±0.34	0	6.47±0.68	5.62±0.69	0	5.88±1.49	6.12±1.23	0.59	5.65±1.081	4.03±1.53	0
Cu	11.91±0.88	11.05±1.07	0.01	16.91±0.38	16.52±0.31	0	40.52±10.71	24.64±5.91	0	11.35±1.37	10.58±1.50	0.10
Zn	21.73±2.41	20.23±2.38	0.06	20.70±1.02	19.60±0.84	0	25.25±1.84	24.86±1.89	0.52	28.43±1.27	26.28±1.22	0
Pb	1.50±0.33	1.09±0.50	0	2.49±0.30	1.87±0.47	0	2.16±0.16	1.47±0.54	0	0.54±0.15	0.40±0.17	0.01
Fe	77.90±9.61	68.27±9.70	0	71.56±11.98	65.31±7.18	0.05	167.13±9.47	149.90±14.25	0	47.33±5.37	45.82±3.90	0.32
Mg	133.32±2.28	131.48±1.26	0	132.54±0.91	131.76±0.90	0.01	133.36±0.26	132.67±0.26	0	131.86±0.48	131.37±0.53	0.004
Mn	4.29±0.23	3.93±0.11	0	4.66±0.87	4.42±0.99	0.42	8.49±0.88	5.46±1.72	0	4.63±0.59	3.88±0.24	0
Ni	4.48±1.73	2.05±1.10	0	3.56±2.15	2.89±1.71	0.28	3.12±1.39	2.26±1.07	0.04	0.73±0.49	0.80±0.33	0.61

Values are mean ±SD; Means in the column are statistically significantly different at (P<0.05).

Table 2: Comparative mean ± SD values of Cr, Cu, Zn, Pb, Fe, Mg, Mn and Ni (mg/L) in intestine, kidney, liver and of muscles of cow from two different sampling sites of study area

Name of metal	Intestine			Kidney			Liver			Muscles		
	Site 1	Site 2	P-Value	Site 1	Site 2	P-Value	Site 1	Site 2	P-Value	Site 1	Site 2	P-Value
Cr	0.82±0.41	0.82±0.36	0.99	3.76±.74857	3.30±0.71	0.05	3.54±0.94	2.62±0.23	0	3.86±1.20	3.60±0.84	0.43
Cu	8.83±1.66	5.44±0.90	0	6.31±0.96	4.83±1.03	0	25.96±0.57	25.22±0.79	0.002	6.65±0.54	5.38±0.37	0
Zn	25.02±1.18	22.95±1.42	0	25.03±1.52	22.90±1.86	0	36.72±0.75	35±0.20	0	25.87±4.50	19.94±1.23	0
Pb	2.49±0.15	2.32±0.20	0	1.98±0.54	1.78±0.73	0.33	1.18±0.50	0.81±0.63	0.04	0.87±0.39	0.59±0.25	0.01
Fe	83.29±3.05	79.02±1.35	0	104.27±10.17	97.86±11.64	0.07	63.32±1.91	61.96±1.79	0.02	46.85±4.80	40.22±0.29	0
Mg	134.57±1.75	132.22±1.67	0	124.38±4.16	23.73±4.45	0.64	130.45±0.36	130.39±0.33	0.60	135.74±0.93	133.90±0.63	0
Mn	2.30±0.64	1.99±0.57	0.12	3.24±0.38	2.85±0.34	0	1.70±0.50	1.54±0.30	0.24	5.09±0.11	4.80±0.08	0
Ni	7.29±0.93	6.04±0.50	0	1.79±1.10	0.57±0.29	0	5.98±0.08	6.07±0.05	0	4.86±0.22	4.60±0.3	0.005

Values are mean ±SD; Means in the column are statistically significantly different at (P<0.05).

Fe, Mg and Ni in intestine of slaughtered cow from site 1 and site 2 were significantly (P<0.05) different from each other. While non-significant difference was observed in case of Cr and Mn in intestine of slaughtered cow collected from sampling sites 1 and 2. Enteritis was grossly noted in sample of selected animals. In histopathological study, most of the intestinal sections of buffalo and cow were showing the more infiltration of inflammatory cells in samples collected from site1 as compared to site 2. Infiltration of inflammatory cells in intestine of buffalo taken from sampling site1 is shown in Fig. 1.

Kidney: The presence of heavy metals in human food chain affects the consumer health. Concentration of Cr, Cu, Zn, Pb and Mg (mg/L**)** in kidney of slaughtered buffalo of sampling site 1 showed significant difference (P<0.05) from samples of site 2 While Fe, Mn and Ni were non-significant (Table 1). Concentrations (mg/L) of Cu, Zn, Mn and Ni in kidney of slaughtered cow from site 1 and site 2 were significantly (P<0.05) different from each other. While non-significant difference was observed in case Cr, Pb, Fe and Mg concentration in kidney of slaughtered cow collected from sampling site 1 and site 2 (Table 2). However, the levels of Cr and Pb in kidney samples of cows taken from both sites were significantly higher than the permissible levels given by Food and Agriculture Organization (FAO) i.e 1 mg/kg and 0.5mg/kg respectively. Kidneys were looked normal but few samples were grossly swollen. Histopathological findings of kidneys sections revealed lesions like shrinkage and congestion in glomerulus. In peri-glomerular area, there was necrosis and degeneration of renal tubules and infiltration of mononuclear inflammatory cell in that tubular area. There was a focal area in the renal cortex where there was necrosis and degeneration and infiltration of large number of mononuclear inflammatory cells. Perivascular cuffing (infiltration of inflammatory cells around blood vessel) was also seen. Heavy infiltrations of mononuclear inflammatory cells were found in all selected sections of buffalos and cows of site 1 as compared to site 2. Kidney sections of site 1 were having giant cells too. Some lesions of kidney are shown in Fig. 2.

Liver: Cu, Pb, Fe, Mg, Mn and Ni (mg/L**)** residues in liver of slaughtered buffalo of selected site 1 showed significant difference (P<0.05) than site 2 while Cr and Zn were non-significant (Table 1). Concentrations (mg/L) of Cr, Cu, Zn, Pb, Fe and Ni in liver of slaughtered cow from site 1 and site 2 were significantly (P<0.05) different from each other. The concentrations of Cr and Pb in all liver samples were higher than acceptable levels as described by FAO. While non-significant difference was observed regarding concentration of Mg and Mn in liver of slaughtered cow collected from sampling site 1 and site 2 (Table 2). Grossly liver of animals showed necrotic focci and redness. In liver sections, congestion in sinusoidal capillaries, hydropic degeneration and cellular swelling in hepatocytes, focal area of infiltration of inflammatory cells (lymphocytes) were observed. Some infiltration was seen in interlobular septum as a result the septum is thick. Few changes in the liver section have been shown in Fig. 3.

Muscle: The concentrations of Cr, Zn, Pb, Mg and Mn (mg/L**)** in muscle of slaughtered buffalo of sampling site1 and site 2 showed significant difference (P<0.05) while Cu, Fe and Ni were differing non-significantly (Table 1). It is proved from table 2 that concentrations (mg/L) of Cu, Zn, Pb, Fe, Mg, Mn and Ni in muscle of slaughtered cow from site 1 and site 2 were significantly (P<0.05) different from each other. While non-significant difference was observed for Cr in muscle of slaughtered cow collected from sampling sites 1 and 2. Grossly muscles were normal. The lesions in some muscles section of slaughtered buffalo and cow were hemorrhages, loss of sarcoplasm, degeneration and necrosis in muscles fibers as shown in Fig. 4. Lesions were more pronounced in sections taken from samples of site 1 as compared to site 2.

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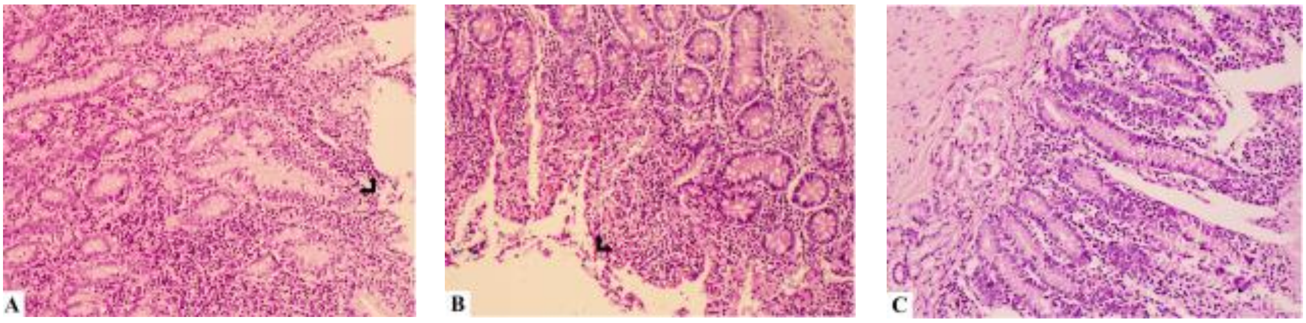

Fig. 1 A: Photomicrograph of intestine of buffalo from site 1. Intestine is showing infiltration ↲ of mononuclear cells in selected area (H&E; 10). **B:** Photomicrograph of intestine of cow from site 1. Intestine is showing infiltration of mononuclear inflammatory cells ↲ in selected area (H&E; 10). **C:** Photomicrograph of intestine of cow from site 2. Selected area of intestine is normal (H&E; 10).

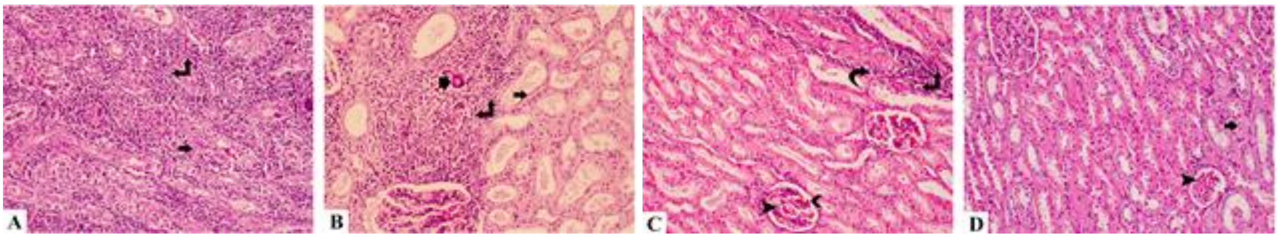

Fig. 2: A: Photomicrograph of kidney of buffalo of site 1. Total selected area is showing the heavy infiltration ↲ of mononuclear inflammatory cells. Degeneration ➡ and necrosis of renal tubules is also seen in this area of kidney (H&E; 10). **B:** Photomicrograph of kidney of buffalo from site 1. kidney is showing infiltration ↲ of mononuclear inflammatory cells in intestitium as well as in glomerulus area and degeneration ➡ of renal tubular area. Giant cell ➧ is also present (H&E; 10). **C:** Photomicrograph of kidney from buffalo of site 2. kidney is showing shrinkage ❯ of glomeruli, congestion in glomerulus ➤, peri-vascular coffin ↱ (infiltration inflammatory cells around blood vessels) and excessive infiltration ↲ (H&E; 10). **D:** Photomicrograph of kidney of cow from site 2. Kidney is showing necrosis ➡ in tubules and congestion ➤ in glomerulus (H&E; 10).

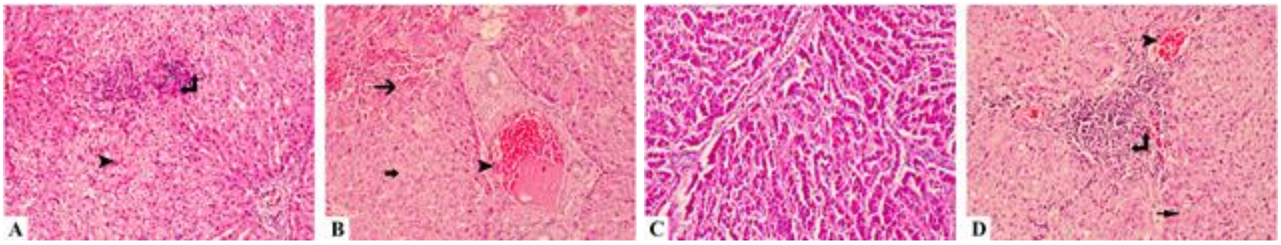

Fig. 3: A: Photomicrograph of liver of buffalo from site 1. Liver is showing congestion ➤ in sinusoidal capillaries, hydropic degeneration in hepatocytes and focal area of infiltration ↲ of inflammatory cells (H&E; 10). **B:** Photomicrograph of liver of cow from site 1. Selected area of liver is showing degeneration ➡ of hepatic cord, hemorrhages → and congestion ➤ (H&E; 10). **C:** Photomicrograph of liver of cow from site 2. Liver is normal (H&E; 10). **D:** Photomicrograph of liver of buffalo from site 2. Liver is showing infiltration ↲ of mononuclear inflammatory cells, hydropic degeneration —► and congestion ➤ (H&E;10).

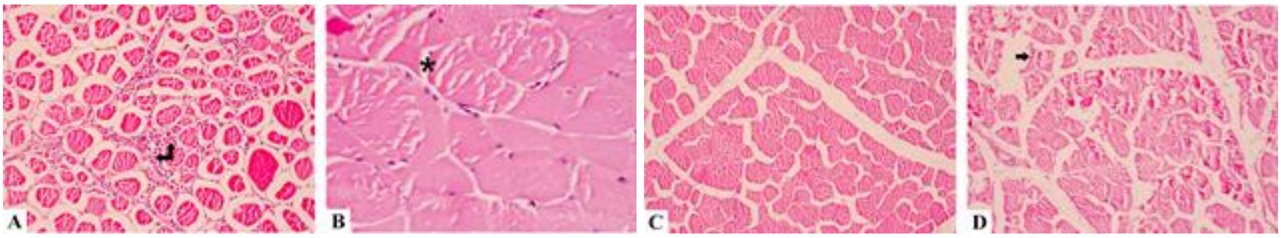

Fig. 4: A: Photomicrograph of muscle of buffalo from site 1. Muscle show infiltration ↲ of mononuclear cells. (H&E; 10). **B:** Photomicrograph of muscle of cow from site 1. Selected area of muscle is showing fragmentation and loss of sarcoplasm * (H&E; 40). **C:** Photomicrograph of muscle of buffalo from site 2. No changes shown in muscle (H&E; 10). **D:** Photomicrograph of muscle of buffalo from site 2. Some degeneration ➡ shown in muscle (H&E; 10).

DISCUSSION

In the recent times, different advancements are being carried out in various fields of science and technology which results in hasty industrialization. In developing countries, this change is much faster than the developed regions of the world (Zhu, 2013). Non-observance of standard protocols for management of toxic industrial effluent is a major problem leading to contamination of drinking water and fodder irrigated with this waste which may ultimately results in high levels of heavy metals in fodder and in animal's body. Heavy metals can be found in living organisms but they become highly toxic when the permissible limit is crossed. They can act as a catalyst to accelerate the working of different enzymes at normal concentration but when their concentration exceeds from normal, adverse metabolic reactions may occur. Heavy metals which are highly toxic to the body include Lead, Nickel, Copper, Zinc, Manganese, Iron and Chromium. They can disturb the normal functioning of certain enzymes by forming complexes. In present study, residual concentration of heavy metals and histopathological alterations in different organs of cows and buffaloes raised on contaminated water and fodder were determined. Two sites were selected near industrial main drain where animals were present and they were reared on the contaminated fodder and water. Residual concentration of heavy metals (Cr, Cu, Zn, Pb, Fe, Mg and Mn) in intestine, kidney, liver and muscle of animals from site 1 were higher than site 2 except Ni. Results of this study were similar to the finding of Bala *et al.* (2014). They determined the levels of Pb, Cr and Cd in liver, kidney, muscles and blood of slaughtered animals depicting highly contaminated environment leading to bio-accumulation of heavy metals. A similar study was also conducted by Sajid *et al*. (2018). Where heavy metal accumulation in the organs was evident in sheep of the area of industrial drain. The results of that study are also in line with current study where similar high concentration of heavy metals in different organs of cows and buffalo was observed. In the present study concentration of heavy metals were comparatively high in the samples collected from the animals of site 1 which was near the drain. Various other studies on cattle, calves and camels (Abdou *et al.*, 2015; Elsayed *et al*., 2015; Khalafalla *et al*., 2015; Seiyaboh *et al.*, 2018) also support the findings of current study that environmental, water and fodder contamination with industrial waste leads to enhanced accumulation of heavy metals in the body of the animals which may ultimately affect human. Abalaka (2015) determined the levels of heavy metals in fish collected from dam to assess the level of contamination of dam water. The results were in agreement of present research that elevated levels of heavy metals were reported in fish organs as well as in dam water.

Histopathological alterations evident from this study were also supported by study of Kaur *et al*. (2018) i.e. Infiltration of inflammatory cells in intestine, necrosis and degeneration of renal tubules, and infiltration of mononuclear inflammatory cell in tubular area were observed in the organs having elevated levels of heavy metals. The changes in intestine and kidney are indicative of exposure to toxic chemical substances and heavy metals leading to cellular damage (Abalaka, 2015). While changes in liver sections of present research i.e. degeneration, cellular swelling in hepatocytes and focal area of inflammatory cells infiltration (lymphocytes) were similar with the findings of Mahmoud and Rahman (2017) and Kar *et al*. (2018). Cellular infiltrations and necrotic changes in cells depict inflammatory responses in the affected liver which might be the consequence of the toxic effects of the metals. Above mentioned liver lesions were not specific to pollutants. A relationship was existed between metal concentrations and liver lesions. The more residual concentration of metal, the more severe was the damage. The histopathological vagaries in organs were more pronounced in the samples taken from site 1 of the study area indicating more residual levels due to more contamination. However, as compared to liver, kidney and intestine, muscles showed little degenerative changes. The histopathological changes observed in present study were also comparable to the previous research by Gholami *et al*. (2004) who observed same lesions in cattle which was heavily intoxicated by heavy metal. Lemos *et al*. (2004) also reported similar findings in case of lead poisoning in cattle.

Conclusions: High levels of heavy metals can cause toxic effects in animals affecting their edible parts and meat, thus entering the heavy metals in the food chain which may seriously harm the human health if not controlled. Therefore, necessary steps should be taken to minimize the contamination of heavy metals in forages, crops and animal products.

Authors contribution: SS, MR, AA, and MR designed the experiment and substantially contributed to analysis and evaluation of clinical data and wrote the manuscript. All authors read and approved the manuscript.

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