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Detection of Selected Heavy Metals and Their Effect on Haematological and Serum Biochemical Values in Naturally Exposed Cattle

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ABSTRACT

Purpose: *The present study was conducted in two areas (one clean and one contaminated) of the district of Jhang, Pakistan, to determine the concentration of the possible heavy metals, lead and cadmium, in the blood. For this purpose, two hundred samples were collected, one hundred from each area.*

Research Method: *These samples were analyzed for lead and cadmium using the atomic absorption spectrophotometric method. The haematological parameters packed cell volume, total erythrocyte count, Haemoglobin, total leucocyte count, differential leukocyte count, and erythrocyte sedimentation rate were measured to evaluate the effect of heavy metals on these parameters. A serological study was also conducted to observe its effects on alanine transaminase, aspartate transaminase, urea, and creatinine.*

Findings: *The results showed that the mean values of lead were significantly higher (P<0.05) in contaminated areas compared to clean areas, while the cadmium level was below the detection limit. The mean values of red blood cells, total leucocyte count, Haemoglobin, packed cell volume, and erythrocyte sedimentation rate showed statistically non-significant differences. The mean values of alanine transaminase were statistically higher in contaminated areas as compared to clean areas, whereas the levels of aspartate transaminase, urea, and creatinine showed non-significant differences in both areas. The presence of lead above permissible levels is alarming from one health point of view, so there is a dire need for legislation to control this transmission of heavy metals to humans and animals.*

Keywords: *Cadmium, Cattle, Haematology, Lead, Serology.*

INTRODUCTION

The metal, which is toxic at low concentrations and has comparatively high density, is called heavy metal, i.e., lead (Pb), mercury (Hg), zinc (Zn), copper (Cu), etc. Some of them are important to sustain the metabolism of the body, like Zn and Cu, and they may become toxic if their concentration increases in the body, while others are not required in the body and cause health hazards (Panday *et al.*, 2014). Toxicity usually develops as a result of unnecessary accumulation of metals in the body through ingestion or inhalation. The long-term ingestion of toxic metals develops accumulative effects on organs and is a major concern of toxicity to animals and humans. Pb and Cd are known for causing illness with pathological effects on the nervous system, kidney, blood, and bone and causing cardiovascular diseases, carcinogenesis, and leukaemia (Akoto *et al.*, 2014).

Due to increasing trends of industrialization and urbanization, Pb and Cd contamination is increasing day by day. Even the rural areas are highly populated and sources of generation of Pb and Cd particles in household wastes. There is no mechanism for the processing of household wastes, and unprocessed sewerage water is being used for irrigation.

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The agricultural and animal products are containing higher levels of Pb and Cd, which are posing threats to human and animal health. Consumers must be aware of the metal particles, and there is a dire need to educate people about the dangerous effects of heavy metals on health. Pb is a major source of harm to domesticated creatures all over the domain. It has spread at large scales in the environment, animals, water, plants, soil, air, and man-made constructions by human beings. It can be postulated that the majority of the population will be exposed to Pb particles as higher Pb residues are present in the environment and products (Siddiqui *et al.*, 2008).

The Cd is also a toxic heavy metal and interacts with the activity of Zn in the enzymatic system (Satarug *et al.*, 2010). It is released from different sources like industrial effluents, agricultural wastes, and mining activities. When these residues rush into water, they cause very threatening problems to the aquatic life (Burger *et al.*, 2008). The long-time exposure to Cd causes nephrotoxicity, lung cancer, pulmonary obstruction, heart disorders, and problems of bones (Pandey *et al.*, 2014).

Cattle are a major source of production of milk and meat all over the world. They usually depend on grazing around canals, riverbanks, and sewerage drains. The grazing animals have more chances of ingestion of metals, including Pb and Cd (Ihedioha *et al.*, 2012). The ingested metals are usually absorbed from the reticulum part of the stomach and accumulate in different edible tissues of the body. The metal accumulation in tissues depends on the concentration of metals in forage, water, and dose; the period of exposure; and the age of animals (Alonso *et al.*, 2004).

Higher levels of Pb and Cd in milk of animals have been reported, and the reason was supposed to be the higher metal levels in water and forage (Ul Haq *et al.*, 2011; Sajid *et al.*, 2017). The higher metal level in meat was demonstrated either due to cumulative absorption of metal in different organs from contaminated forage and drinking water or contamination in dirty slaughterhouses (Kazzah *et al.*, 2020; Karimi *et al.*, 2020). Lead (Pb) and cadmium (Cd) were present in both pasteurized and raw milk of goat (Javed *et al.*, 2009). Keeping in view this scenario, the present study was planned to investigate the status of Pb and Cd in our local cattle along with their effects on haematological and biochemical parameters in Jhang, Pakistan.

MATERIALS AND METHODS

Selection of Location and Animals

The Jhang area was selected because it is fertile and rich in livestock population. It is suitable for crops as it is situated at the union of two rivers (Chenab and Jhelum). Most of the cattle graze in riverine areas, riverbanks, canal banks, and sewerage drains, which are usually contaminated and a source of Pb and Cd. Therefore, the animals were divided into two groups, i.e., grazing in the clean areas, including river areas, riverbanks, and canal banks, and contaminated areas, including the sewerage drain and apparent discolorations in soil and strong odors. A total of 200 adult cattle aged 2-5 years and weighing 350-400kg were selected. These animals were regularly dewormed to prevent parasitic infestation and were very active.

Collection of blood samples

Blood samples (10 mL) were collected from the jugular vein of selected cattle in a sterilized syringe from the jugular vein. 5 mL of blood was poured into a vacutainer tube with EDTA while the 5 mL of blood was poured into a gel-coated vacutainer tube to separate serum. All blood and serum samples were transferred to the Pathology lab CVAS Jhang at refrigerated temperature.

Detection of heavy metals

Heavy metals, including lead (Pb) and cadmium (Cd), were determined in serum samples by atomic absorption spectrophotometric method (Licata *et al.*, 2004). The standards of each heavy metal were run with a blank to obtain the ingested values of elements from samples. A correction curvature was designed to get the final values.

Effect of heavy metals on haematological Parameters

Haematological parameters included packed cell volume (PCV %) total erythrocyte count (RBC x 10^6 /µL), hemoglobin estimation (Hb g/dL), total leukocyte count (TLC x x10^3/µL), differential leukocyte count, and erythrocyte sedimentation rate (ESR mm/hr.) were performed according to our standard lab procedures.

Effect of heavy metals on serological values

Serum samples were subjected to liver and kidney function tests, including ALT, AST, creatinine, and urea

Table 1: The concentration (mg/L) of Pb and Cd in serum of cattle from clean area (SJ) and sewerage drain area (CK)

Metal	Parameter	Clean Area	Sewerage Area
Pb	Mean ± SE	0.338±0.22[a]	0.87±0.120[b]
	Minimum value	ND	ND
	Maximum value	0.36[a]	1.96[b]
Cd	Mean ± SE	ND	ND
	Minimum value	ND	ND
	Maximum value	ND	ND

*Values are Mean ± S.E. The mean values in the row bearing unlike letters (*a,b*) are statistically different at (P<0.05).*

Table 2: The values of haematological parameters of cattle from clean area and sewerage drain area

Parameter	Clean Area	Sewerage Area
RBC	7.575±1.339[a]	6.89±1.339[a]
TLC	7.44±2.259[a]	9.12±2.259[a]
Hb	8.630±1.919[a]	7.873±1.830[a]
PCV	32.435±3.902[a]	31.265±3.786[a]
ESR	2.496±0.0321[a]	2.486±0.0321[a]
Lymphocytes	43.82±22.94[a]	53.18±20.94[a]
Monocytes	6.8±2.33[a]	8.34±3.14[a]
Neutrophils	37.540±8.53[a]	49.62±19.45[a]

Values are Mean ± S.E, the means in the row bearing unlike letters in superscript (a, b) are statistically meaningfully different at (P<0.05).

Table 3: The values of serological parameters of cattle from clean area and sewerage drain area

Parameter	Clean Area	Sewerage Area
ALT	54.5±15.338[a]	90.9±24.881[b]
	16.00–251.00*	21.00–298.00*
AST	41.5±7.124[a]	46.6±7.726[a]
	16.00–116.00*	19.00–119.00*
Urea	27.2±2.008[a]	33.0±2.095[a]
	19.00–39.00	19.00–54.00
Creatinine	0.83±0.0369[a]	0.90±0.0371[a]
	0.40–1.00*	0.60–1.10

*Values are Mean ± S.E, the mean values in the row bearing unlike letters in superscript (a, b) are statistically different at (P<0.05). * Indicates the standard values in cats.*

levels by standard kits according to the manufacturer's instructions.

RESULTS AND DISCUSSION

The mean values of Pb were significantly higher (P<0.05) in the contaminated area as compared to the clean area. The minimum values of Pb were zero (not detectable), while the maximum value of Pb was 0.36 mg/L in the clean area and 1.96 mg/L in the contaminated area. The Cd concentration was below the detection level in both study areas, as depicted in Table 01.

The values of haematological parameters are given in Table 02. The values of RBC, TLC, Hb, PCV and ESR were showing statistically non-significant differences. But the contaminated area showed lower values of RBC, haemoglobin concentration and packed cell volume as compared to clean area whereas the total leukocyte count was higher in contaminated area as compared to clean area of this study. The mean values of lymphocytes, neutrophils and monocytes were comparatively higher in contaminated area as compared to clean area but this difference was also statistically non-significant in both areas.

The mean values of ALT were statistically higher (P<0.05) in contaminated area as compared to clean area whereas the values of AST, urea and creatinine were higher in contaminated areas but showed non-significant difference as shown in Table 03.

The reference range of blood Pb concentration in animals is 0.05–0.25 mg/L of blood. According to the literature, the ruminants show the signs of poisoning at $\geq$0.35 mg/L of blood Pb concentration and death at 1.00 mg/L. Our findings suggested that the mean values of serum Pb concentration in cattle were above the maximum permissible limits of 0.25 mg/L in both areas under study. The cattle under this study were not observed clinically for any signs of Pb toxicity. This indicates that our local cattle are resistant to Pb residue levels above the permissible limits given in literature. High tolerance of our cattle for Pb is supported by the findings of other workers who found that buffaloes possessed high tolerance to Pb poisoning as compared to cattle (Radostits *et al.*, 2007). The similar findings of higher Pb concentration in blood were reported by a researcher in cattle grazing in a mining area, but the clinical observations were not included in their study (Chirinos-Peinado *et al.*, 2020). In accordance with our findings, many researchers detected higher levels of Pb from the blood of ruminants grazing in contaminated areas (Sajid *et al.*, 2017; Rodríguez-Estival *et al.*, 2012; Swarup *et al.*, 2005). Some workers detected higher Cd levels in animals and animal products, which were contrary to our findings. This difference may be due to different animals, area variation, and environment.

In our investigation, the contaminated area had higher Pb levels in cattle (CK). The earlier studies conducted by additional researchers in the same district revealed greater Pb concentrations in sheep serum in the contaminated area (Sajid *et al.*, 2017). Another Greek researcher discovered greater Pb levels in the blood of calves raised close to Pb-Zn foundries; Pb levels were also higher in Pb-Zn smelters; however, only cadmium levels were higher in the vicinity of metal packing factories and steel-working areas (Radostits *et al.*, 2007). Numerous studies found increased amounts of Pb in the blood of ruminants grazing in contaminated areas, which is consistent with our findings (Sajid *et al.*, 2017; Rodríguez-Estival *et al.*, 2012; Swarup *et al.*, 2005). Researchers' findings (Ihedioha *et al.*, 2012; Obeid *et al.*, 2016; Bazargani-Gilani *et al.*, 2016) that found greater Pb levels in meat and edible offals of various animals from slaughterhouses concur with our findings.

In contrast to our findings, where Cd residues were not found in cow serum, the same workers found increased Cd levels in animals and animal products. As we employ serum for the detection of metal residues in our research, this variance could be the result of different animals, geographic variation, or tissue differences. Low Pb and Cd levels were discovered in the blood of male and female cattle from slaughterhouses in a different study by a researcher. A study from Slovakia found that grazing sheep had blood with greater Pb and lower Cd concentrations, which supports our findings for cattle raised in contaminated areas.

In contrast to our results, researchers (Sajid *et al.*, 2017; Alabbassi *et al.*, 2008) observed higher neutrophil counts in different studies that might be due to different animals or environmental conditions. Our findings showed no significant difference in lymphocyte and eosinophil counts, which are concurrent with the results of Sajid *et al.* (2017) and Alabbassi *et al.* (2008), who observed higher lymphocytes and low eosinophils in their studies. This difference may be due to different animals or different areas of study. The higher levels of TLC of our research in cattle of the contaminated area were similar to the findings of Zaki *et al.* (2010), who reported higher TLC in Marino sheep grazing in the contaminated area. The findings of Prakash *et al.* (2009) also supported our results of higher TLC due to Pb toxicity.

The liver is the main organ that shows changes in acute Pb toxicity (Farrag *et al.*, 2007), and higher levels of ALT and AST are indicators of liver damage in live animals. Our findings of higher ALT levels in cattle are consistent with Akan *et al.* (2010), who demonstrated severe liver damage in Pb and Cd toxicity. In our findings, the values of urea and creatinine are statistically non-significantly different in both clean and contaminated areas. The same findings were reported by a worker (Sajid *et al.*, 2017) in sheep, where urea concentration was non-significant, but in contrast to our findings, the creatinine level was significantly higher, which might be due to breed variation in different locations of the study area.

CONCLUSION

It was concluded that higher levels of lead (Pb) are present in the blood of cattle in both areas, especially in contaminated areas. It interferes with the haematological values, and kidney and liver functions. The animals do not show any signs of toxicity, indicating an alarming situation because this can be transferred to humans through animal products. This study will suggest that higher authorities adopt effective measures to control the higher levels of these metals and standardize their levels in different animals and animal products.

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