Original Article

A Study of Heavy Metal Status and its Relationship with Hematologic and Biochemical Indices in River Buffaloes in Southwest Iran

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ABSTRACT

Heavy metals are among the most important environmental pollutants which accumulate in various organs and are associated with several toxic effects. This study was performed to determine the status of heavy metals in river buffaloes in Khuzestan province, Iran, and its relationship with hematologic and serum biochemical parameters. A total of 103 apparently healthy buffaloes were sampled from the region. The concentration of heavy metals, including lead (Pb), mercury (Hg), and cadmium (Cd), was determined in serum samples by atomic spectroscopy. In addition, complete blood counts and serum biochemical profiles were assessed. The serum concentration of Cd, Pb, and Hg in the sampled buffaloes, as mean±standard error, were 0.55±0.01, 6.51±0.10, and 6.28±0.09 µg/l, respectively, which are within the permissible serum levels in the livestock. Serum Cd and Hg levels showed no significant relationship with hematologic or biochemical analytes. However, there were significant negative correlations between Pb levels and phosphorus, magnesium, sodium, as well as potassium concentrations, while serum iron was positively correlated with lead (P<0.05). In addition, there was a significant positive correlation between Hg level and serum aspartate aminotransferase activity (P<0.05). Despite the fact that river buffaloes in Khuzestan spend a long time daily in the Karun River with high industrial pollution, no serum evidence of heavy metal toxicity was found in these animals. It can be suggested that river buffaloes in Khuzestan seem to be resistant to the environmental pollution caused by heavy metals. However, further studies are required to confirm this issue and identify its possible explanations.

Keywords: Biochemistry, Bubalus bubalis, Heavy metal, Hematology

1. Introduction

Despite its numerous benefits, the development of the industry has led to certain problems, such as environmental pollution. Heavy metals are among the most critical environmental pollutants that can continue their cyclic motion in the environment for a long time due to their ability to accumulate in various tissues, as well as their non-degradability and resistance to biological changes. They are gradually stored in the tissues of consumers and thus cause many acute or chronic toxic risks. Among the heavy metals, lead (Pb), cadmium (Cd), and mercury (Hg) are of great importance due to their harmful effects on human and animal health, as well as their ability to be transmitted through the food chain (1).

Exposure to these elements can lead to toxic complications in various organs, including renal toxicity and failure, liver failure, and bone marrow hematopoietic tissue damage (2).

River buffaloes (Bubalus bubalis) are economically and culturally valuable livestock in Iran. There are three main river buffalo breeds in Iran: Azeri, Khuzestani, and Mazandarani, with 119000, 81000, and 4000 individuals, respectively. The Khuzestani is reared in the west and southwest (mainly in the Khuzestan province) of the country and raised outdoors throughout the year. Iranian buffaloes are mostly kept for their milk production which is richer in protein, fat, lactose, and energy, compared to cattle. Water buffaloes provide about 293,000 tons of milk (2.8% of Iran's total milk production) and 24,700 tons (2.5%) of meat (3). Their unique adaptability to unstable conditions in terms of temperature and moisture, their resistance to diseases and parasites, as well as their long productive life, have made buffaloes remarkable species.

Due to the characteristics and behaviors of river buffaloes, water availability is important to them, especially in hot climates, since they need rivers or splashing water to assist in thermoregulation (4).

One of the most central water resources in Khuzestan is the Karun River, in the basin of which there are several industrial factories that, in addition to consuming the water, discharge their industrial wastewater into the river. Furthermore, this river is considered the recipient of sewage and effluents of hospitals and agricultural drains that are the primary sources of heavy metals. Other sources include soil, as well as forage in industrial areas and adjacent to major roads (1).

Considering the breeding of river buffaloes along the Karun River, the possibility of contamination of this livestock with heavy metals increases. This contamination is important both in terms of livestock production and human health, as well as animal health risks and toxicity in various organs.

A limited number of studies have been conducted on heavy metal contamination and its consequences on the health of buffaloes and cattle in other regions of the world, especially Pakistan, India, and Mexico, which mostly included anemia, kidney, and liver dysfunction, as well as oxidative damage (5). However, no documented investigation has been performed on Khuzestan river buffaloes in this regard.

Khuzestan river buffaloes are raised beside the Karun River, as a recipient of industrial wastewater, which raises the potential of heavy metal contamination and the uncertainty of the degree of these contaminations in this livestock. Therefore, the present study aimed to determine serum concentrations of heavy metals in buffaloes in Khuzestan province, Iran, to indicate their level of contamination with metal elements and its relationship with hematologic and serum biochemical parameters. It also aimed to identify the toxicity of heavy metals in livestock health, as well as the development of methods to prevent buffalo exposure to these environmental pollutants.

2. Materials and Methods

2.1. Sampling Animals

This study was conducted on a 103 sample of apparently healthy buffaloes (approved by clinical examinations), 36 females and 67 males, ranging in age from 1 to 10 years, slaughtered in Ahvaz abattoir. Animals with cachexia, fever, diarrhea, and respiratory symptoms were excluded from this study. After recording the signalments (gender and age) of the studied animals, blood samples were taken from the jugular vein and collected in two tubes with and without anticoagulant (EDTA) for hematologic examination and serum isolation, respectively. Serum samples were stored in a -20˚C freezer until testing.

2.2. Serum Heavy Metal Measurement

Determination of heavy metals, including Pb, Hg, and Cd, in serum samples was performed by the Central Laboratory of *** (the university title was anonymized) using Atomic Spectroscopy (ICP-OES) (Optima-8300 PerkinElmer, Waltham, MA, USA).

2.3. Hematologic Assessment

Complete blood counts, including total erythrocyte count (RBC), hematocrit value (HCT), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total white blood cells were determined by the BC-2800Vet hematology analyzer (Mindray, China).

Whole blood samples were also used to prepare thin blood smears for microscopic examination. Blood smears were stained with 5% Giemsa solution following fixation with methanol. The prepared smears were then examined in terms of erythrocyte morphologic abnormalities related to heavy metal toxicity, most commonly basophilic stippling. More than 30 microscopic fields of blood films were examined under a ×100 objective lens. Approximately 20,000 erythrocytes were carefully searched per slide. Differential leukocyte counts were also estimated microscopically (6).

2.4. Serum Biochemical Assessment

Serum biochemical parameters, including total protein, albumin, urea, iron (Fe), total bilirubin, total cholesterol, calcium (Ca), phosphorus (P), and magnesium (Mg) concentrations, aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were assessed spectrophotometrically with a biochemistry autoanalyzer (BT-1500, Biotecnica, Italy) using commercial colorimetric kits (Parsazmun, Iran) (7). The serum concentration of electrolytes, including sodium (Na) and potassium (K), were also measured by flame photometry (Sherwood Scientific, England).

2.5. Statistical Analysis

The statistical analysis was performed using the SPSS software (version 16, SPSS Inc., Chicago, IL, USA). Power analysis was performed, and the required sample size was determined accordingly. The distribution of the data was tested by the Shapiro-Wilk test, which revealed a normal distribution. The Independent sample t-test was used to compare and determine statistical differences in laboratory-obtained values between male and female groups. In addition, the one-way analysis of variance (ANOVA), followed by the Tukey Post Hoc test, was performed to compare the data between groups, classified based on various heavy metal levels and animal age. Furthermore, the correlation between variables was determined by the Pearson correlation coefficient. All values were expressed as mean±standard error, and $P<0.05$ was considered statistically significant.

3. Results

3.1. General Assessment

The mean serum concentrations of Cd, Pb, and Hg are demonstrated in table 1.

Table 1. Serum heavy metal concentrations (mean ± SE) assessed in river buffaloes in Khouzestan

Metal	n	Mean±SE	Range
Cd (µg/l)	103	0.55 ± 0.01	0.18 -0.99
Pb (µg/l)	103	6.51 ± 0.10	4.59 – 8.95
Hg (µg/l)	103	6.28 ± 0.09	4.39 – 8.83

Cd: cadmium, Pb: lead, Hg: mercury

3.2. Blood Smear Examination

The assessment of blood smears did not reveal any heavy metal-related morphologic changes in erythrocytes (including basophilic stippling).

3.3. Effect of Gender

The mean serum level of Hg was significantly higher in male buffaloes (n=67) than in females (n=36, P=0.002). However, there were no significant differences in serum Cd and Pb concentrations between the two genders (P>0.05) (Table 2).

Table 2. Mean ± SE of serum cadmium, lead, and mercury in male and female river buffaloes

	Female (n=36)	Male (n=67)
Cd (µg/l)	0.53 ± 0.02	0.56 ±0.02
Pb (µg/l)	6.38 ± 0.17	6.58 ± 0.13
Hg (µg/l)	5.90 ± 0.14*	6.50 ± 0.11

Asterisk (*) indicates significant difference between male and female in each row (P<0.05)
Cd: cadmium, Pb: lead, Hg: mercury

3.4. Effect of Age

There was no significant difference in any of the assessed heavy metal serum levels in various age groups (Table 3). However, the mean MCV was significantly higher in those 4-5 and 5< years, compared to buffaloes aged 2> and 2-3 years (P<0.05). In addition, there was a significant rise in serum cholesterol levels in buffaloes of above 5 years, compared to those 2> and 2-3 years (P<0.05).

3.5. Effect of Cadmium Levels on Hematologic and Serum Biochemical Parameters

Hematologic and biochemical data were classified and compared based on serum Cd concentration in three groups of ≤0.4 µg/l (n=15), 0.4<-≤0.6 µg/l (n=56), and 0.6< µg/l (n=32) (Table 4). The statistical analysis revealed that despite some minor variations, there was no significant difference in hematologic and biochemical parameters between various groups.

3.6. Effect of Lead Levels on Hematologic and Serum Biochemical Parameters

Samples were divided into three groups based on serum Pb concentration: ≤5.7 µg/l (n=29), 5.7 <-≤7 µg/l (n=40), and >7 µg/l (n=34). The lowest values of serum P and Na concentrations were in group 3 (>7 µg/l), which were significantly lower than the other groups (P<0.05) (Table 5). Furthermore, the maximum serum Fe concentration was for group 3, which was significantly higher than the other groups (P<0.05).

3.7. Effect of Mercury Levels on Hematologic and Serum Biochemical Parameters

Data were divided into three groups according to serum Hg: ≤5.7 µg/l (n=33), 5.7<-≤6.5 µg/l (n=34), and >6.5 µg/l (n=36). The comparison between groups in terms of hematologic and biochemical results indicated the absence of any statistically significant differences (Table 6).

3.8. Relationship between Heavy Metal Concentrations and Hematologic and Biochemical Indicators

The correlation between hematologic and biochemical data and the level of heavy metals was investigated (Table 7). The results expressed significant negative correlations of Pb levels with P, Mg, Na, and K concentrations, while serum Fe was positively correlated with Pb (P<0.05). In addition, there was a significant positive correlation between Hg levels and AST activity (P<0.05).

Table 3. Mean ± SE of metal, hematologic and serum biochemical parameters in various age groups of river buffaloes

	Age (year)				
	2>	**2-3**	**3-4**	**4-5**	**>5**
Cd (µg/l)	0.57 ± 0.04	0.56 ± 0.03	0.54 ± 0.04	0.60 ± 0.06	0.52 ± 0.03
Pb (µg/l)	6.34 ± 0.24	6.56 ± 0.17	6.67 ± 0.36	6.60 ± 0.40	6.28 ± 0.16
Hg (µg/l)	6.36 ± 0.35	6.37 ± 0.13	6.48 ± 0.37	6.73 ± 0.35	6.03 ± 0.15
WBC ($\times10^3$/µl)	9.44 ± 0.87	9.33 ± 0.88	8.95 ± 0.99	8.03 ± 0.81	9.21 ± 0.86
Neut ($\times10^3$/µl)	3.86 ± 0.44	4.13 ± 0.43	4.76 ± 0.72	4.44 ± 0.56	4.50 ± 0.42
Lym ($\times10^3$/µl)	5.53 ± 0.76	4.49 ± 0.37	4.03 ± 0.54	3.63 ± 0.58	4.49 ± 0.52
Eos ($\times10^3$/µl)	0.02 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.04	0.16 ± 0.04
Mon ($\times10^3$/µl)	0.09 ± 0.08	0.06 ± 0.03	0.11 ± 0.05	0.09 ± 0.08	0.04 ± 0.02
RBC ($\times10^6$/µl)	8.36 ± 0.87	7.90 ± 0.38	8.44 ± 0.050	7.14 ± 0.59	7.08 ± 0.25
Hb (g/dl)	12.12 ± 1.93	11.42 ± 0.66	12.27 ± 0.70	11.04 ± 0.83	10.79 ± 0.47
HCT (%)	42.84 ± 5.50	40.85 ± 2.21	46.15 ± 2.61	41.69 ± 3.20	40.09 ± 1.35
MCV (fl)	50.51 ± 1.40 [a*]	52.09 ± 0.88 [a]	55.05 ± 2.13 [ab]	58.84 ± 2.33 [b]	56.91 ± 1.04 [b]
MCH (pg)	13.99 ± 0.77	14.46 ± 0.27	14.55 ± 0.49	15.55 ± 0.59	15.15 ± 0.29
MCHC (%)	27.63 ± 0.69	27.39 ± 0.51	26.54 ± 0.25	26.50 ± 0.30	26.73 ± 0.35
RDW (%)	16.75 ± 0.61	16.17 ± 0.16	15.85 ± 0.21	16.07 ± 0.59	16.04 ± 0.25
PLT ($\times10^3$/µl)	122.09 ± 15.85	138.08 ± 11.12	170.44 ± 15.94	126.43 ± 27.61	164.59 ± 14.28
Urea (mg/dl)	27.90 ± 2.42	33.41 ± 1.46	33.66 ± 3.90	30.14 ± 3.16	34.79 ± 2.24
Pro (g/dl)	6.42 ± 0.23	6.67 ± 0.18	6.55 ± 0.20	7.08 ± 0.25	6.80 ± 0.17
Alb (g/dl)	3.40 ± 0.06	3.67 ± 0.16	3.38 ± 0.17	3.53 ± 0.12	3.39 ± 0.07
Total Bil (mg/dl)	0.71 ± 0.03	0.75 ± 0.01	0.79 ± 0.05	0.77 ± 0.05	0.75 ± 0.03
Chol (mg/dl)	60.45 ± 5.73 [a]	57.45 ± 3.32 [a]	53.83 ± 12.93 [ab]	53.86 ± 8.58 [ab]	45.39 ± 4.89 [b]
ALP (U/l)	317.64 ± 20.06	265.85 ± 16.33	321.22 ± 43.26	238.14 ± 50.29	254.62 ± 25.84
AST (U/l)	23.25 ± 6.10	37.44 ± 7.20	58.22 ± 35.95	15.28 ± 5.42	26.83 ± 5.91
Ca (mg/dl)	9.24 ± 0.10	8.95 ± 0.11	8.82 ± 0.35	9.14 ± 0.18	8.93 ± 0.10
Pho (mg/dl)	7.25 ± 0.30	6.42 ± 0.31	6.18 ± 0.59	6.60 ± 0.35	6.27 ± 0.19
Iron (µg/dl)	60.27 ± 7.99	63.90 ± 4.08	45.52 ± 17.50	59.83 ± 6.09	64.18 ± 5.33
Mg (mg/dl)	3.54 ± 0.32	4.04 ± 0.23	3.39 ± 0.45	3.57 ± 0.19	4.04 ± 0.23
Na (mEq/l)	134.82 ± 3.08	141.42 ± 3.83	148.11 ± 14.55	129.57 ± 4.21	140.00 ± 2.75
K (mEq/l)	10.14 ± 0.64	9.79 ± 0.73	9.23 ± 2.35	8.11 ± 1.20	9.43 ± 0.52

* Different superscript letters in each row represent significant difference between groups (P<0.05).
Cd: cadmium, Pb: lead, Hg: mercury, WBC: white blood cells, Neut: neutrophils, Lym: lymphocytes, Eos: eosinophils, Mon: monocytes, RBC: red blood cells, Hb: hemoglobin concentration, HCT: hematocrite value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: rec cell distribution width, PLT: platelet count, Pro: total protein, Alb: albumin, Total Bil: total bilirubin, Chol: total cholesterol, ALP: alkaline phosphatase activity, AST: aspartate aminotransferase activity, Ca: Calcium, P: Phosphorus, and Mg: Magnesium, Na: Sodium, and K: Potassium

Table 4. Mean ± SE of hematologic and serum biochemical parameters in various levels of cadmium contamination in river buffaloes

	Cd (µg/l)			Reference Interval (8, 9)
	≤0.4 (n=15)	**0.4<-≤0.6 (n=56)**	**0.6<(n=32)**	
WBC ($\times10^3$/µl)	10.4 ± 1.76	9.47 ± 0.70	8.77 ± 0.73	4.00 – 12.00
Neut ($\times10^3$/µl)	4.49 ± 0.89	4.41 ± 0.30	4.33 ± 0.42	0.60 – 4.00
Lym ($\times10^3$/µl)	5.70 ± 0.97	4.54 ± 0.39	4.33 ± 0.39	2.50 – 7.50
Eos ($\times10^3$/µl)	0.04 ± 0.02	0.09 ± 0.03	0.08 ± 0.02	0.00 – 0.24
Mon ($\times10^3$/µl)	0.04 ± 0.02	0.08 ± 0.02	0.05 ± 0.03	0.08 – 0.84
RBC ($\times10^6$/µl)	6.85 ± 0.37	7.81 ± 0.29	7.74 ± 0.21	5.00 – 10.00
Hb (g/dl)	10.2 ± 0.55	11.57 ± 0.56	11.65 ± 0.65	8.00 – 15.00
HCT (%)	38.6 ± 2.12	41.68 ± 1.74	42.99 ± 2.12	24.00 – 46.00
MCV (fl)	56.53 ± 1.85	53.64 ± 0.86	54.24 ± 0.99	40.00 – 60.00
MCH (pg)	14.87 ± 0.43	14.75 ± 0.26	14.54 ± 0.29	11.00 – 17.00
MCHC (%)	26.4 ± 0.2	27.27 ± 0.37	26.92 ± 0.35	30.00 – 36.00
RDW (%)	16.06 ± 0.29	16.44 ± 0.21	15.83 ± 0.15	-
PLT ($\times10^3$/µl)	135.16 ± 31.85	144.07 ± 8.47	159.65 ± 10.54	-
Urea (mg/dl)	35.58 ± 2.37	33.51 ± 1.43	30.8 ± 1.94	42.80 – 64.20
Pro (g/dl)	6.58 ± 0.37	6.73 ± 0.12	6.69 ± 0.15	6.74 – 8.12
Alb (g/dl)	3.76 ± 0.36	3.49 ± 0.08	3.39 ± 0.05	3.03 – 3.55
Total Bil (mg/dl)	0.71 ± 0.02	0.75 ± 0.02	0.76 ± 0.02	1.00 – 2.00
Chol (mg/dl)	43.36 ± 8.26	55.14 ± 2.88	53.74 ± 4.81	80.00 – 120.00
ALP (U/l)	221.66 ± 23.55	276.1 ± 14.7	281.8 ± 25.33	0.00 – 488.00
AST (U/l)	58.25 ± 15.98	24.21 ± 3.78	34.06 ± 11.55	78.00 – 132.00
Ca (mg/dl)	9.05 ± 0.13	8.96 ± 0.10	9.00 ± 0.07	9.70 – 12.40
Pho (mg/dl)	6.61 ± 0.5	6.5 ± 0.22	6.42 ± 0.21	5.60 – 6.50
Iron (µg/dl)	52.6 ± 6.19	63.95 ± 4.13	59.53 ± 4.99	57.00 – 162.00
Mg (mg/dl)	2.6 ± 0.42	1.84 ± 0.15	1.67 ± 0.24	1.80 – 2.30
Na (mEq/l)	141.25 ± 5.89	141.02 ± 3.65	135.06 ± 2.24	132.00 – 152.00
K (mEq/l)	5.7 ± 0.76	4.56 ± 0.65	3.93 ± 0.49	3.90 – 5.80

Cd: cadmium, WBC: white blood cells, Neut: neutrophils, Lym: lymphocytes, Eos: eosinophils, Mon: monocytes, RBC: red blood cells, Hb: hemoglobin concentration, HCT: hematocrite value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: rec cell distribution width, PLT: platelet count, Pro: total protein, Alb: albumin, Total Bil: total bilirubin, Chol: total cholesterol, ALP: alkaline phosphatase activity, AST: aspartate aminotransferase activity, Ca: Calcium, P: Phosphorus, and Mg: Magnesium, Na: Sodium, and K: Potassium

Table 5. Mean ± SE of hematologic and biochemical parameters in various levels of lead contamination in river buffaloes

	Pb (µg/l)			Reference Interval (8, 9)
	≤5.7 (n=29)	**5.7<-≤7 (n=40)**	**7< (n=34)**	
WBC ($\times10^3$/µl)	9.15 ± 0.77	8.34 ± 0.52	10.83 ± 1.19	4.00 – 12.00
Neut ($\times10^3$/µl)	4.69 ± 0.46	3.67 ± 0.27	5.11 ± 0.51	0.60 – 4.00
Lym ($\times10^3$/µl)	4.30 ± 0.45	4.46 ± 0.35	5.07 ± 0.64	2.50 – 7.50
Eos ($\times10^3$/µl)	0.05 ± 0.02	0.10 ± 0.03	0.08 ± 0.03	0.00 – 0.24
Mon ($\times10^3$/µl)	0.04 ± 0.01	0.08 ± 0.03	0.09 ± 0.04	0.08 – 0.84
RBC ($\times10^6$/µl)	7.58 ± 0.38	7.8 ± 0.36	7.78 ± 0.35	5.00 – 10.00
Hb (g/dl)	11.08 ± 0.54	11.58 ± 0.66	11.45 ± 0.67	8.00 – 15.00
HCT (%)	41.16 ± 1.99	42.49 ± 2.04	41.06 ± 2.1	24.00 – 46.00
MCV (fl)	54.55 ± 1.16	54.68 ± 0.9	53.36 ± 1.2	40.00 – 60.00
MCH (pg)	14.63 ± 0.32	14.71 ± 0.29	14.74 ± 0.32	11.00 – 17.00
MCHC (%)	26.88 ± 0.23	27.01 ± 0.39	27.22 ± 0.48	30.00 – 36.00
RDW (%)	15.71 ± 0.22	16.26 ± 0.22	16.47 ± 0.23	-
PLT ($\times10^3$/µl)	139.59 ± 12.13	142.51 ± 11.24	160.35 ± 12.75	-
Urea (mg/dl)	34.23 ± 2.07	32.74 ± 1.38	32.19 ± 2.17	42.80 – 64.20
Pro (g/dl)	6.79 ± 0.24	6.71 ± 0.13	6.63 ± 0.14	6.74 – 8.12
Alb (g/dl)	3.64 ± 0.16	3.53 ±0.12	3.36 ± 0.07	3.03 – 3.55
Total Bil (mg/dl)	0.75 ± 0.02	0.74 ± 0.02	0.76 ± 0.01	1.00 – 2.00
Chol (mg/dl)	54.95 ± 4.87	50.03 ± 4.12	55.89 ± 3.76	80.00 – 120.00
ALP (U/l)	277.32 ± 27.03	281.82 ± 17.65	253.22 ± 20.13	0.00 – 488.00
AST (U/l)	33.63 ± 15.53	32.79 ± 6.02	29.28 ± 5.7	78.00 – 132.00
Ca (mg/dl)	8.99 ± 0.08	9.11 ± 0.06	8.84 ± 0.16	9.70 – 12.40
Pho (mg/dl)	6.85 ± 0.22	6.82 ± 0.21	5.84 ± 0.29	5.60 – 6.50
Iron (µg/dl)	49.65 ± 4.67	60.5 ± 3.89	69.79 ± 5.8	57.00 – 162.00
Mg (mg/dl)	2.31 ± 0.26	1.94 ± 0.22	2.51 ± 0.17	1.80 – 2.30
Na (mEq/l)	153.41 ± 7.08	136.41 ± 2.47	132.35 ± 2.07	132.00 – 152.00
K (mEq/l)	5.89 ± 1.06	4.55 ± 0.48	4.5 ± 0.4	3.90 – 5.80

Pb: lead, WBC: white blood cells, Neut: neutrophils, Lym: lymphocytes, Eos: eosinophils, Mon: monocytes, RBC: red blood cells, Hb: hemoglobin concentration, HCT: hematocrite value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: rec cell distribution width, PLT: platelet count, Pro: total protein, Alb: albumin, Total Bil: total bilirubin, Chol: total cholesterol, ALP: alkaline phosphatase activity, AST: aspartate aminotransferase activity, Ca: Calcium, P: Phosphorus, and Mg: Magnesium, Na: Sodium, and K: Potassium

Table 6. Mean ± SE of hematologic and biochemical parameters in various levels of mercury contamination in river buffaloes

	Hg (µg/l)			Reference Interval (8, 9)
	≤5.7 (n=33)	5.7<-≤6.5 (n=34)	6.5< (n=36)	
WBC ($\times10^3$/µl)	10.2 ± 1.13	7.9 ± 0.53	9.92 ± 0.83	4.00 – 12.00
Neut ($\times10^3$/µl)	4.48 ± 0.47	3.66 ± 0.27	4.66 ± 0.44	0.60 – 4.00
Lym ($\times10^3$/µl)	4.57 ± 0.58	4.15 ± 0.34	5.06 ± 0.50	2.50 – 7.50
Eos ($\times10^3$/µl)	0.08 ± 0.03	0.08 ± 0.03	0.09 ± 0.03	0.00 – 0.24
Mon ($\times10^3$/µl)	0.04 ± 0.02	0.09 ± 0.05	0.07 ± 0.03	0.08 – 0.84
RBC ($\times10^6$/µl)	7.19 ± 0.33	8.31 ± 0.45	7.72 ± 0.31	5.00 – 10.00
Hb (g/dl)	10.62 ± 0.52	12.58 ± 0.92	11.09 ± 0.46	8.00 – 15.00
HCT (%)	38.78 ± 1.88	44.86 ± 2.54	41.4 ± 1.77	24.00 – 46.00
MCV (fl)	54.88 ± 1.27	54.13 ± 0.91	53.73 ± 1.05	40.00 – 60.00
MCH (pg)	14.97 ± 0.28	14.87 ± 0.37	14.34 ± 0.27	11.00 – 17.00
MCHC (%)	26.86 ± 0.5	27.54 ± 0.53	26.79 ± 0.19	30.00 – 36.00
RDW (%)	16.32 ± 0.21	16.17 ± 0.23	16.13 ± 0.25	-
PLT ($\times10^3$/µl)	166.14 ± 14.89	143.59 ± 9.5	136.68 ± 11.55	-
Urea (mg/dl)	33.75 ± 2.01	32.17 ± 1.59	32.85 ± 1.87	42.80 – 64.20
Pro (g/dl)	6.69 ± 0.19	6.64 ± 0.16	6.75 ± 0.15	6.74 – 8.12
Alb (g/dl)	3.31 ± 0.08	3.58 ± 0.14	3.57 ± 0.11	3.03 – 3.55
Total Bil (mg/dl)	0.74 ± 0.03	0.75 ± 0.01	0.75 ± 0.02	1.00 – 2.00
Chol (mg/dl)	47.6 ± 4.13	58.0 ± 3.84	53.06 ± 4.54	80.00 – 120.00
ALP (U/l)	270.18 ± 20.14	264.97 ± 21.77	276.6 ± 20.4	0.00 – 488.00
AST (U/l)	21.96 ± 5.11	35.95 ± 5.82	36.06 ± 11.03	78.00 – 132.00
Ca (mg/dl)	8.83 ± 0.14	8.99 ± 0.08	9.11 ± 0.1	9.70 – 12.40
Pho (mg/dl)	6.34 ± 0.23	6.61 ± 0.28	6.49 ± 0.26	5.60 – 6.50
Iron (µg/dl)	57.3 ± 6.55	64.07 ± 4.36	61.17 ± 4.4	57.00 – 162.00
Mg (mg/dl)	1.86 ± 0.24	1.9 ± 0.21	1.89 ± 0.22	1.80 – 2.30
Na (mEq/l)	141.57 ± 4.08	134.33 ± 2.24	141.29 ± 4.66	132.00 – 152.00
K (mEq/l)	4.81 ± 0.69	4.55 ± 0.53	4.23 ± 0.8	3.90 – 5.80

Hg: mercury, WBC: white blood cells, Neut: neutrophils, Lym: lymphocytes, Eos: eosinophils, Mon: monocytes, RBC: red blood cells, Hb: hemoglobin concentration, HCT: hematocrite value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: rec cell distribution width, PLT: platelet count, Pro: total protein, Alb: albumin, Total Bil: total bilirubin, Chol: total cholesterol, ALP: alkaline phosphatase activity, AST: aspartate aminotransferase activity, Ca: Calcium, P: Phosphorus, and Mg: Magnesium, Na: Sodium, and K: Potassium

Table 7. Correlation of cadmium, lead, and mercury with hematologic and biochemical parameters in river buffaloes

	Cd (µg/l)	Pb (µg/l)	Hg (µg/l)
	Pearson correlation	**Pearson correlation**	**Pearson correlation**
WBC ($\times10^3$/µl)	-0.138	0.202	0.005
Neut ($\times10^3$/µl)	-0.068	0.150	0.034
Lym ($\times10^3$/µl)	-0.168	0.180	0.041
Eos ($\times10^3$/µl)	-0.002	-0.021	0.051
Mon ($\times10^3$/µl)	-0.072	0.137	-0.004
RBC ($\times10^6$/µl)	0.088	0.069	0.073
Hb (g/dl)	0.049	0.061	-0.011
HCT (%)	0.053	0.019	0.013
MCV (fl)	-0.071	-0.091	-0.171
MCH (pg)	-0.061	0.019	-0.204
MCHC (%)	0.014	0.093	0.007
RDW (%)	-0.154	0.160	0.040
PLT ($\times10^3$/µl)	0.045	0.122	-107
Urea (mg/dl)	-0.111	-0.017	0.026
Pro (g/dl)	0.003	-0.081	-0.009
Alb (g/dl)	-0.104	-0.113	0.139
Total Bil (mg/dl)	0.047	0.087	0.134
Chol (mg/dl)	0.106	0.044	0.080
ALP (U/l)	0.046	-0.094	0.061
AST (U/l)	-0.047	-0.057	0.234*
Ca (mg/dl)	0.009	-0.102	0.176
Pho (mg/dl)	-0.007	-0.265*	0.031
Iron (µg/dl)	0.072	0.233*	0.077
Mg (mg/dl)	-0.099	-0.243*	-0.058
Na (mEq/l)	-0.142	-0.310*	0.005
K (mEq/l)	-0.078	-0.241*	-0.070
Age	-0.116	-0.121	-0.109

Asterisk (*) indicates significant correlation between intersecting parameters (P<0.05)
Cd: cadmium, Pb: lead, Hg: mercury, WBC: white blood cells, Neut: neutrophils, Lym: lymphocytes, Eos: eosinophils, Mon: monocytes, RBC: red blood cells, Hb: hemoglobin concentration, HCT: hematocrite value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: rec cell distribution width, PLT: platelet count, Pro: total protein, Alb: albumin, Total Bil: total bilirubin, Chol: total cholesterol, ALP: alkaline phosphatase activity, AST: aspartate aminotransferase activity, Ca: Calcium, P: Phosphorus, and Mg: Magnesium, Na: Sodium, and K: Potassium

4. Discussion

Environmental pollution is a global problem, and heavy metals are one of the most central pollutants. Karun River pollution with heavy metals has been proven and documented since long ago due to the discharge of industrial and agricultural wastewater into this river (10, 11). Considering the breeding of river buffaloes in Khuzestan along the Karun River, this study was conducted to investigate the status of heavy metal contamination in this species and its effects on laboratory indicators of animal health.

Heavy metal assessment in serum can be a significant means to detect exposure to environmental pollutants (12). In this study, the concentrations of Cd, Pb, and Hg were measured in buffalo serum at 0.55±0.01, 6.51±0.10, and 6.28±0.09 µg/l (ppb), respectively.

Blood Pb concentrations are an excellent marker of exposure in animals. The normal background concentration of Pb in the blood of cows is below 0.1 mg/l (ppm), while Pb concentrations of above 0.35 ppm are compatible with a diagnosis of Pb toxicosis in clinically affected animals (12). Additionally, Shailaja, Reddy (13) reported much higher levels of Pb (25-95 µg/dl) than the current study in river buffalo serum in the Hyderabad region of India. Consequently, the mean serum Pb level of the studied buffaloes (6.51±0.10

µg/l) was much lower than the toxic levels previously published (12).

In addition, high blood Cd concentrations have been reported from areas with high Cd exposure. In India, cows reared and kept near a steel manufacturing plant had a mean blood Cd concentration of 232 µg/l (ranging from 90 to 410 µg/l). Comparatively, a mean blood Cd concentration of 28 µg/l (ranging from non-detectable to 50 µg/l) was measured in cows from a non-polluted area (14). Therefore, the mean serum Cd concentration in the currently studied buffaloes (0.55±0.01 µg/l) was in the normal range, compared to the previously reported levels in cattle and buffaloes in non-polluted areas.

So far, little information has been documented on livestock serum Hg levels in polluted and non-polluted areas. Nevertheless, Hg concentrations of <0.1 mg/kg (weight) are considered normal in many body organs, including blood in most species, and concentrations of >6 mg/kg (blood) are consistent with a diagnosis of poisoning (15). Therefore, in the case of Hg, similar to other elements analyzed in the present study, the measured values in the blood of buffaloes (6.28±0.09 µg/l) were in the normal (non-contaminated) range.

It seems that despite the type of habitat, and especially the exposure of the region's buffaloes to the Karun River, which contains a high level of sewage pollution and industrial and domestic waste, the level of absorption and accumulation of heavy metals is not significant in this species, compared to that reported in previous studies (15). Compatibly, Orjales, Herrero-Latorre (16) compared the status of essential and toxic trace elements in organic and conventional dairy cattle. The mean concentration of serum Cd, Hg, and Pb in organic farms was 0.294, 0.122, and 1.175 µg/l, respectively, while in the conventional system, it was 0.162, 0.139, and 0.842 µg/l, respectively, in the summer (16). Moreover, in a recent study, plastic foreign body impaction in buffaloes was associated with higher levels of heavy metals, including Hg, Cd, and Pb in serum, body fluids, and tissues; however, it was below the toxic levels (17).

Considering the difference between males and females in the present study, a significant difference was only observed in the concentration of Hg so that the serum level of this element was significantly higher in male buffaloes than females. However, in the case of other measured metals, no significant difference was observed between the two genders. Similarly, Bazargani-Gilani, Pajohi-Alamoti (18) examined heavy metals and traced elements in the kidneys and livers of cattle, sheep, and slaughtered goats, and the results showed no significant difference in the concentration of heavy metals in bovine samples in terms of gender and age. The lower Hg level in females might be probably due to the physiological characteristics of female animals, the reproductive cycles, and pregnancy, which prevent the absorption or help to excrete more of this element. Comparably, in a study performed by Khan, Ugulu (19), blood Cd level was lower in lactating compared to non-lactating buffaloes.

The age of animals did not affect the serum levels of Cd, Pb, and Hg in the current study. The only significant difference was in MCV and cholesterol, which were higher in older buffaloes, compared to younger ones. Considering the almost constant metal levels in young and mature animals, these variations might be due to the exclusive effect of age. Moreover, studies have already reported an increase in both MCV and total cholesterol with the age of buffaloes (20).

There was no significant difference in terms of hematologic and biochemical parameters between the groups with different levels of Cd in this study. Furthermore, no significant correlation was observed between the Cd level and serum biochemical analytes. However, Barrasso, Ceci (21) detected Cd residues in the muscle, liver, and kidney of river buffaloes slaughtered in Italy. Tissue Cd levels that exceeded the limits imposed by European regulations were associated with typical histopathologic lesions in kidney samples (21). It seems that the amount of Cd in the body of animals in the present study was lower than the toxic level and had no recognizable effect on the health status or function of various organs.

Hg levels also had no significant effect on laboratory health indicators of buffaloes. Although there was a positive correlation between AST enzyme activity and blood Hg concentration, the range of activity of this enzyme in the studied animals was in the normal interval, and this relationship did not seem to be clinically considerable. Conversely, Mahajan, Yadav (22) reported a significant decline in Hb level and a significant rise in blood urea nitrogen, serum glutamate oxaloacetate transaminase, albumin, and creatinine in cattle exposed to Hg from fly ash due to rearing within 5 km of a thermal power station. The mean serum Hg concentration in the exposed cattle group was recorded at 7.41 mg/kg (22). Moreover, it has been shown that higher serum Hg and Pb in the spring were accompanied by lower hematocrit, Hb, and erythrocyte count in sheep (2). Overall, the level of exposure to Hg in this study does not appear to affect the health of buffaloes in terms of hematologic and biochemical markers.

There was no considerable relationship between blood Pb concentrations and laboratory analytes. While there was a negative correlation between Pb and P, Mg, Na, as well as K levels, and a positive correlation between Pb and blood Fe concentration, most of the changes were within the normal range and not associated with alterations in other laboratory parameters; therefore, they do not appear significant. The negative correlation between serum Pb level and P level may be due to the inhibitory effect of Pb on 1α-hydroxylase in renal tubules and, as a consequence, the inhibition of the synthesis of calcitriol resulting in a decrease in P absorption in the intestine (23). It sounds that the amount of Pb exposure of buffaloes in this study, similar to Cd and Hg, was too low to induce any significant changes in the general fitness of animals, which can be represented by the laboratory results. The inverse relationship of Pb with serum Na and K levels is probably due to the increased renal excretion of these electrolytes due to Pb exposure. A possible reason for co-occurring changes in serum Pb and Fe levels might be simultaneous higher exposure of Pb-exposed buffaloes to other metallic elements, including Fe.

However, a higher level of Pb is likely associated with more significant changes in hematologic parameters, as well as the levels of minerals and electrolytes. An experiment on water buffalo calves revealed significant declines in Hb, HCT, RBC, and plasma calcium, following Pb acetate oral administration at a dose of 9.2 mg/kg once daily for 90 days (24). Likewise, Mohajeri, Norouzian (25) reported that the higher level of Pb in dairy cows reared in areas around a Pb-zinc (Zn) smelter was associated with lower hematocrit and Hb, and it also increased the activity of serum transaminase enzymes, compared to the reference group. In addition, in a study conducted by Shailaja, Reddy (13), a negative relationship was recorded between Pb content and serum Mg concentrations in river buffaloes, although this relationship was not statistically significant. Likewise, Ikechukwu, Ojareva (26) reported a negative correlation between blood Pb concentration and serum P, as well as Ca, in pregnant women. Furthermore, kidney and liver serum markers (creatinine, alanine transaminase, and alkaline phosphatase) were significantly elevated in buffaloes with higher serum Pb levels. However, very high levels of serum Pb in buffaloes were associated with decreased levels of Fe and Zn in the blood (26). The general negative association of serum minerals and electrolytes with blood Pb levels in this study might be attributed to the Pb-induced minor decline of mineral availability and/or the role of essential elements in minimizing the absorption of this heavy metal.

In general, Khuzestan buffaloes are in constant contact with the Karun River and regularly consume water from it. According to the records and published references, the Karun River is highly polluted with heavy metals. However, contrary to expectations, the level of heavy metals in the studied animals was relatively low. One of the possible reasons for the absence of a significant relationship between

hematologic and biochemical indices of blood and the levels of heavy metals in the blood of buffaloes in this study was the low level of contamination with these metals in the investigated animals. Given that this is a preliminary study, at this stage, it is not possible to determine the cause of this issue. However, it can be hypothesized that the results are related to two possible phenomena. Firstly, considering that the involvement of Khuzestan river buffaloes in many infectious diseases, including piroplasmosis, is less, and they show relative resistance (still under investigation), there is a possibility of their resistance to some toxic factors, including heavy metals. Secondly, it should also be noted that in the present study, it was not possible to sample hard tissues. Due to the fact that the status of some elements, especially in cases of chronic exposure in storage tissues is more accurate, to clarify the issue, it is better to examine the status of heavy metals in hard tissue and hair samples in future studies.

Additionally, experimental studies, the exposure of river buffaloes to controlled amounts of metal elements, as well as the investigation of their absorption and its effects on animal health, can probably provide valuable information about the reaction of this species to heavy metals.

Despite the fact that Khuzestan buffaloes spend a long time daily in the Karun River and the pre-existing proven industrial pollution of the river, unexpectedly, no serum evidence of heavy metal toxicity was found in the river buffaloes. It can be suggested that the river buffaloes in Khuzestan seem to be resistant to environmental pollution caused by heavy metals. Further studies, including the assessment of heavy metals in hard tissues, are required to confirm this issue and identify its possible explanations.

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Authors' Contribution

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Ethics

All the animal experiments were approved by the Institutional Animal Care and Use Committee of Shahid Chamran University of Ahvaz, and were implemented in strict accordance with approved guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

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