

TOXIC EFFECTS OF HEAVY METALS ON HEALTH AND PRODUCTIVITY OF LIVESTOCK IN LITTLE AKAKI RIVER CATCHMENT, CENTRAL ETHIOPIA

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ABSTRACT

Contaminated water and feed are the main causes of heavy metal poisoning in livestock. Heavy metals such as chromium (Cr), cadmium (Cd), lead (Pb), and zinc (Zn) enter the food chain and can bioaccumulate in the body, leading to chronic toxicity in livestock. In Ethiopia, investigations showed the concentrations of heavy metals in water, soil and vegetables were very high, however, there have been hardly any studies on animals so far. From December 2022 to June 2023, a cross-sectional study was conducted to investigate the toxic effect of heavy metals on the health of livestock in the little Akaki River catchment. Heavy metals from water and blood were analysed using atomic absorption spectrophotometry (AAS) and toxicity levels in livestock were assessed using haematological and biochemical measurements. The result was presented as the mean \pm SD, and statistical association was determined at $P \leq 0.05$. From analysis of water sample, the concentration (mg/L) of Cr, Cd, Pb and Zn were obtained to be 0.009–0.05, 0.003–0.009, 0.09–0.29, and 0.029–0.94 respectively. Cd and Pb were found to be above the permissible limit (Cd= 0.003 and Pb = 0.015) set by FAO/WHO for livestock drinking. From analysis of blood samples the concentrations (mg/L) of Cr, Cd, and Pb were higher in villages 1 and 2 as compared to village 3, which was statistically significant ($p < 0.05$). The mean concentration (mg/L) of Cd (0.009 in Village-1 and 0.0083 in Village-2) and the mean concentration (mg/L) of lead (0.16 in Village-1 and 0.154 in Village-2) exceeded the minimum allowable limit of 0.007 mg/L for cadmium and 0.02 mg/L for lead. The duration of exposure to heavy metals had a significant impact on its bioaccumulation in the blood ($p < 0.05$). In addition, haematological and biochemical parameters such as packed cell volume, haemoglobin, white blood cell count, urea, total protein, and albumin were significantly ($P < 0.05$) decreased in Village-1 and Village-2, while alkaline phosphatase and aspartate and neutrophil counts were significantly increased in Village-1 and Village-2 compared to Village-3. Therefore, the objective of this study was to determine the toxic effects of heavy metal on the livestock health exposed to the Little Akaki River.

Key words: *Concentration, Heavy metals, Livestock, Little akaki river, Toxicity*

1. INTRODUCTION

1.1. Background

Surface water resources such as rivers, lakes, and streams have been extensively utilized for various purposes, including human and livestock consumption, irrigation, industrial activities, transportation, recreation (Barakat *et al.*, 2016). Water is a crucial component for the survival and proper functioning of all living beings, including animals. It makes up approximately 70-80% of newborns and 65-70% of the weight of adult livestock. This is essential in ensuring optimal milk production, growth rate, resistance against diseases, and successful reproduction in livestock (Lardner *et al.*, 2005; Beede, 2009).

Although water has numerous benefits, human activities have led to water pollution which is a critical environmental concern (Habeeb *et al.*, 2018). Water quality degradation resulting from pollution is a progressively escalating concern in Ethiopia, the magnitude and intensity of which is significantly more pronounced within large urban centers throughout the country particularly in and around the capital city the problem of water pollution is at its highest stage, especially at the little akaki river (Agaje, 2007).

In the recent past, various environmental and human-induced factors are responsible for the contamination of water quality. The unregulated discharge of sewage and industrial wastewaters, along with the inappropriate disposal of waste produced by agriculture and healthcare sectors, have greatly impacted the deterioration of water bodies (Tadesse *et al.*, 2018). Consequently, the river system's quality of water decreases due to the introduction of hazardous substances, such as toxic heavy metals (Deshu *et al.*, 2021).

The little Akaki River contained a large amount of trace metals (Kassegne *et al.*, 2018). However, small scale farmers grow various types of vegetables and depend on the little Akaki River as the main water source for irrigation due to the scarcity of fresh water (Mengesha *et al.*, 2021). Additionally, in the Little Akaki River downstream region, livestock predominantly rely on the river's water resources and are allowed to forage on pastures that may be polluted. As a

result, significant amounts of heavy metals can be transferred to animals directly or indirectly from polluted water sources and subsequently, they can spread through the food chain and pose a major health risk to people consuming the residues in animal products (Umar *et al.*, 2014; Gupta *et al.*, 2021).

1.2.Statement of problem

Various literatures have been published on heavy metal contamination of water, soil and vegetables at different levels of wastewater-irrigated urban farming sites in Akaki River basin and found that concentrations (mg/L) of chromium, lead cadmium, and Zinc metals are higher than the recommended limit by FOA/WHO. However there has been little research on animals so far; therefore, this research also included the effect of the river water on livestock health through analysis of their blood.

1.3.Research hypothesis

Little Akaki River is contaminated by toxic substances that have detrimental effects on the livestock health and productivity.

1.4.General objective

- To determine the toxic effects of heavy metal exposure on the livestock health exposed to the Little Akaki River.

1.5.Specific objectives

- To determine the concentration level of heavy metals (Chromium, Cadmium, Lead and Zinc) in the river water
- To determine the extent of bioaccumulation of heavy metals in the blood of animals
- To measure hematological and biochemical alterations due to heavy metals poisoning

1.6. Scope and limitations of the research

There are many heavy metals but this study is limited to analysis of the heavy metals Cadmium, Lead, Zinc and Chromium from field samples such as river water and blood samples.

1.7. Significance of the study

Livestock are suffering more from consumption of polluted water. Little Akaki river water is used for a variety of purposes from which livestock drinking without prior treatment is common trend. Hence protecting the ecological health of the rivers is vital for the livestock health which is directly or indirectly exposed to the river water. The data that emanated from this research work will have multiple benefits. The data generated are expected to be usable in integrated water management for improved livestock and human health. It is also important in the creation of awareness of decision makers, various stakeholders and the general public about undesirable consequence of improper wastewater management in livestock health. Thus, institutions like Environmental Protection Authority, Ministry of water, Irrigation and Power, Ministry of Agriculture will obviously benefit from the results of the present study.

2. MATERIALS AND METHODS

2.1. Study area

The research was conducted in the Little Akaki River catchment, which drains the Addis Ababa administration and is used for small scale farming and livestock drinking. It is located at varying elevations ranging from 2464 a.s.l at Gefersa reservoir in the north to 2048 a.s.l at Aba Samuel reservoir in the south. The river originates as a small stream emerging from Mount Entoto, which is located in the north-west of the city of Addis Ababa. It then merges with the Geferssa Reservoir, flows through the south-west of the city and finally flows into the Aba Samuel Reservoir after a distance of about 40 km (Worku and Giwtea, 2018). The Little Akaki River has a temperate Afro-Alpine climate. Average annual rainfall is 1254 mm and average daily temperatures range from 9.9 to 24.6 °C. In the lower course of river animals from three villages based on their proximity to the river; Village-1 ('Qersa') and Village-2 ('Ichu') are located 500 m - 1 km from the river and Village-3 (Gelaan-gudda) is > 2 km from the river.

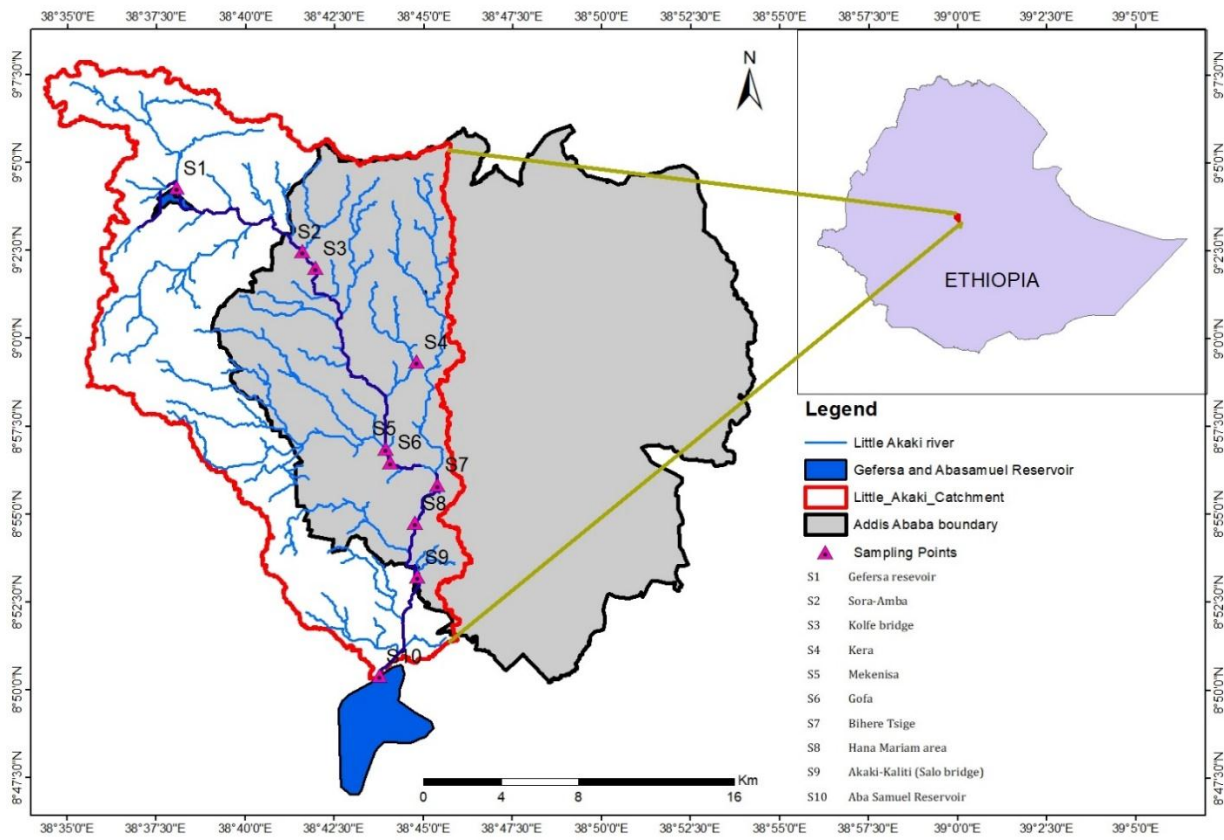


Figure 1. Map of the study area

2.2. Study design

The study was conducted from December 2022 to June 2023 using a cross-sectional study design. After a preliminary investigation in the study areas, water samples were collected from 10 sampling points of the little Akaki River, taking into account accessibility, proximity to point and non-point sources of pollution. In addition, forty (40) animals of different sexes, species and ages were randomly sampled from three villages in the downstream areas of Little Akaki river basin. The studied animals were categorized as young, adult and old based on dentition pattern described by Pace and Wakeman (2003).

2.3. Sampling design

Triplicate water samples were collected from 10 sampling points along the river basin and transported to the laboratory in a pre-washed and cleaned 250 ml plastic bottle for physicochemical analysis and heavy metal analysis. Blood sample from cattle and sheep reared in downstream of little Akaki river catchment was collected for heavy metal analysis, biochemical and hematological tests. Accordingly the blood were collected from jugular vein in EDTA coated and plain vacutainer tube (10ml) then it preserved in ice packed cold box, transported to laboratory until analysis.

2.4. Physico-Chemical analysis of Water

The analysis of the physico-chemical parameters was carried out immediately after delivery to the laboratory. The parameters analyzed include dissolved oxygen (mg/l), electrical conductivity (s/cm), resistivity (m), total dissolved solids (mg/l) and salinity (PSU). They were measured with a multi-parameter instrument (HANNA instruments, model: HI9829-01042). The turbidity (NTU) of the water sample was determined with the instrument (TL2360 LED Turbidimeter, ISO, 0-10000 NTU).

2.5. Heavy metal analysis

Water samples for heavy metal analysis were preserved in 5 mL HNO₃ and stored in the refrigerator at 4 °C to prevent volatilization and biological degeneration between sampling and the analysis period (Weldegebriel *et al.*, 2012b). The blood sample was analyzed for heavy metals immediately after delivery to the laboratory.

2.5.1. Digestion of water sample

Water samples were digested according to established protocols to assess the levels of heavy metals (Cd, Cr, Pb and Zn) (Deshu *et al.*, 2021). The preserved water samples were mixed well and 100 mL of the mixture was then digested in a glass-covered beaker by adding 5 mL of concentrated HNO₃, which is used to dissolve the sample and destroy all of its organic matrix (Mitra and Somenath 2003). Samples were gradually heated on a hot plate at digestion temperatures above 90 °C until the volume was reduced to approximately 15 to 20 mL (Usepa, 1992) for 1 to 2 hours (Kingston and Jassie 1986), depending on specific conditions. After boiling and evaporating the sample, 5 ml of conc. HNO₃ and 2mL H₂O₂ were added to the digest and reheated until the digest became a light and clear solution. Finally the outcrop was cooled, filtered and analyzed by atomic absorption spectroscopy.

2.5.2. Digestion of blood sample

The whole blood was centrifuged. Then 2 mL of blood plasma and 2 mL of H₂SO₄ were combined and the combined sample left in the laboratory overnight to allow digestion. All natural tissues were digested by heating the sample solution to 120°C. To improve digestion by breaking it down at this point, 2mL of H₂O₂ was added. The excess acid mixture, after evaporation, was cooled to a semi-dry mass. The digested samples were diluted with up to 50 mL of distilled water, placed in glass tubes and stored in the refrigerator at 40 °C until analyzed by atomic absorption spectrophotometer (AAS) for analysis (Hussain, *et al.*, 2021).

2.5.3. *Determination chromium, cadmium, lead and zinc in water and blood samples*

Atomic absorption spectrophotometer (Buck Scientific Model 210 VGP AAS, USA) was used to measure the concentration of heavy metals in digested samples. It was adjusted and calibrated in accordance with the manufacturer's instructions. The continuous sample introduction system, which includes an auto-sampler to inject and analyze the sample solution obtained by acid dilution as well as the reference sample, was also used. Finally, the concentrations of each of the heavy metals (Pb, Cd, Cr and Zn) were determined based on spike test and reference samples.

2.5.4. *Preparation of standard reference of heavy metals validation measurements*

To prepare the calibration solutions, nitric acid and internal standards were used to dilute the multi-element standards. The validation measurements of AAS were performed using a working calibration solution of the studied toxic heavy metals (Cd, Cr, Pb and Zn). These solutions were prepared by appropriate stepwise dilution of a certified standard stock solution of the elements (Ultra grade 1000 g/mL, 5% HNO₃, ULTRA scientific analysis solutions) (Luna *et al.*, 2019).

2.6.Hematological and biochemical analysis

The following parameters were calculated from a whole blood sample: hemoglobin, packed cell volume, leukocyte, neutrophil, eosinophil, basophil, monocyte, and lymphocyte levels. Blood biochemical markers such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, and blood urea nitrogen (BUN) were measured using an automated biochemical analyzer (Automatic Biochemical Analyzer, Emp-168) using a commercially available kit.

2.7.Data analysis

The collected data was entered in to Microsoft excel and analyzed using STATA 14 (64-bit). The obtained data were expressed as MEAN \pm standard deviation (SD). The p-value \leq 0.05 were considered as statistically significant. The data was analyzed using t-test and one-way analysis of variance (ANOVA)

3. RESULTS

3.1. Physico-chemical parameters of little akaki river

The mean the temperature of the LAR water samples in the current study ranged from 16.7 ± 1.15 to 23 ± 1.53 °C. The highest temperature prevailed at the sampling location (S3). However, the lowest temperature was measured at the sampling point (S6). The average pH values of the sampling points were between 6.9 ± 0.11 and 8.48 ± 0.21 . The sampling site (S8) recorded the highest value while the sampling site (S2) recorded the lowest pH. The average concentration of DO in LAR was ranged from 3.44 ± 0.34 to 6.46 ± 0.52 mg/L; the highest DO concentration was recorded at sampling site (S5) while the lowest DO was recorded at the sampling site (S9). The average concentration of TDS and salinity of water samples were varied between 82 ± 4.9 to 642 ± 31.4 (mg/l) and 46 ± 6.3 to 139 ± 15.9 (PSU) respectively; the highest concentration of TDS and salinity were recorded at sampling site S8 and S4 respectively while their lowest values were obtained at the sampling site (S1). The average turbidity measurements from sampling sites were ranged from 82 ± 6.8 to 914 ± 55 NTU (Table 1). Variations in the physicochemical characteristics of the river water at each sampling location may be caused by the improper and unregulated disposal of waste from various companies, the healthcare sector, and home and commercial sewerage systems.

Table 1. Physico-chemical parameters of the little akaki river

Param eter	Physicochemical parameters (mean \pm SD)									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Temp. (°c)	22 \pm 1.5	19 \pm 1.53	23 \pm 1.53	19.6 \pm 2.1	19 \pm 1.9	16.7 \pm 1.15	20.8 \pm 1.7	22.7 \pm 1.3	19.6 \pm 1.7	18 \pm 1.5
PH	7.61 \pm 0.21	6.91 \pm 0.11	7.5 \pm 0.05	7.29 \pm 0.08	7.21 -	7.23 \pm 0.06	7.21 \pm 0.13	8.48 \pm 0.21	7.43 \pm 0.2	7.44 \pm 0.3
DO (mg/L)	5.39 \pm 0.51	6.22 \pm 0.1	4.83 \pm 0.04	6.06 \pm 0.74	6.46 \pm 0.52	4.27 \pm 0.1	3.84 \pm 0.4	4.32 \pm 0.5	3.44 \pm 0.34	4.67 \pm 0.33
TDS (mg/L))	82 \pm 4.9	195 \pm 8.7	488 \pm 14.8	515 \pm 7.6	588 \pm 15.5	568 \pm 27.3	566 \pm 28.7	642 \pm 31.4	552 \pm 13	569 \pm 23
Sal. (PSU)	46 \pm 6.3	67 \pm 10.7	89 \pm 13.6	139 \pm 15.9	127 \pm 22	126 \pm 14	131 \pm 20.6	125 \pm 15.9	97 \pm 8	83 \pm 9.5
Turb. (NTU)	82 \pm 6.9	660 \pm 30.7	414 \pm 33.6	379 \pm 63.9	914 \pm 55	910 \pm 68.7	735 \pm 74.7	722 \pm 43.2	811 \pm 26.1	621 \pm 67.5

PH= power of hydrogen, DO= dissolved oxygen, TDS= total dissolved solids

3.2.Heavy metals Concentrations in little Akaki river

The average concentration of Cr, Cd, Pb, and Zn in this study varied from 0.015 to 0.05 mg/L, 0.003 to 0.009 mg/L, 0.09 ± 0.03 to 0.31 mg/L, and 0.029 to 0.94 mg/L respectively (Table 2). Accordingly, the sampling sites (S4, S4 & S9, S9 and S10) were recorded the highest concentrations of Cr, Cd, Pb, and Zn respectively.

Table 2. Heavy metal concentration in little akaki catchment (n = 10 sites)

Heavy metals	Concentration (mean \pm SD) in mg/L									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cr	0.015 \pm 0.005	0.025 \pm 0.005	0.034 -	0.05 -	0.031 -	0.009 \pm 0.01	0.038 -	0.025 -	0.041 \pm 0.006	0.015 \pm 0.006
Cd	0.003 -	0.008	0.005 -	0.0091	0.005	0.007	0.008	0.0042 -	0.0091 -	0.004 -
Pb	0.152 \pm 0.03	0.293 \pm 0.03	0.22 \pm 0.03	0.15 \pm 0.03	0.246 \pm 0.03	0.09 \pm 0.03	0.293 \pm 0.02	0.262 \pm 0.02	0.31 -	0.262 \pm 0.02
Zn	0.073 \pm 0.03	0.094 \pm 0.02	0.118 \pm 0.08	0.24 \pm 0.015	0.221 \pm 0.04	0.0324 \pm 0.02	0.116 \pm 0.01	0.029 \pm 0.007	0.518 \pm 0.014	0.94 \pm 0.019

3.3.Heavy metals concentration in cattle and sheep

The concentration of Cr, Cd and Pb in animal blood from village-1 (Qersa) and village-2 (Ichu-1) showed a significantly ($P < 0.05$) higher concentration than the concentration in animal blood from village-3 (Gelaan-gudda) (Table 3).

Table 3. Concentration of heavy metals in in three villages (n=40)

Study areas	Concentration (Mean \pm SD)			
	Cr	Cd	Pb	Zn
Village-1	0.031 \pm 0.007	0.009 \pm 0.003	0.16 \pm 0.033	1.383 \pm 0.174
Village-2	0.0305 \pm 0.005	0.0083 \pm 0.0027	0.154 \pm 0.023	1.3797 \pm 0.208
Village-3	0.009 \pm 0.002	0.002 \pm 0.0006	0.013 \pm 0.0011	1.1542 \pm 0.178
P value	P < 0.05			

The mean values of PCV, HB and total WBC count in village 1 and 2 were statistically significant ($P < 0.05$) and lower, while the blood neutrophil count in Village-1 and Village-2 was higher than Village-3. The serum biochemical parameters such as BUN, TP, and albumin were significantly ($P < 0.05$) decreased, while AST and ALP activity was significant ($P < 0.05$) and higher in village-1 and village-2 than in Village-3 (Table 4)

Table 4. Hematological and biochemical values measured from cattle and sheep (n=40)

Study areas		Blood Parameters								
		PCV %	HB	WBC ($\times 10^3/\mu\text{L}$)	Neut. %	AST U/L	TP g/l	BUN Mg/l	ALP U/L	Alb. g/l
Village-1	Mean	24	8	4.3	55	65	4.7	52.6	73.7	2.3
	SD	± 5	± 3	± 0.4	± 6.7	± 20	± 1.13	± 7.8	± 14.7	± 0.6
Village-2	Mean	24	8	4.4	53	64.9	5	54.2	75	2.54
	SD	± 4.5	± 2	± 0.44	± 6	± 19.8	± 1.3	± 7.31	± 18	± 0.66
Village-3	Mean	38	13	6.3	50	46	7.6	92.8	41	4.2
	SD	± 1.7	± 0.7	± 0.7	± 2	± 8	± 0.5	± 19.6	± 3.8	± 0.28

$P < 0.05$

AST= Aspartate aminotransferase, ALP= Alkaline phosphate, BUN= Blood urea nitrogen, HB= Hemoglobin, PCV= Packed cell volume, TP= total protein,

In the present study, it was found that chromium, cadmium, zinc and lead concentrations were higher in adult cattle aged 4 to 10 years than in older cattle aged > 10 years and younger cattle aged < 4 years (Table 5). However, there was no statistically significant difference ($P > 0.05$) between the groups.

Table 5. Concentration of heavy metals in cattle according to age (n=26)

Age category (year)	Heavy metals concentration (Mean \pm SD) in mg/L			
	Cr	Cd	Pb	Zn
Young (<4)	0.026 \pm 0.012	0.007 \pm 0.005	0.11 \pm 0.07	0.15 \pm 0.06
Adult (4-10)	0.0291 \pm 0.0098	0.0132	0.16	0.16
Older (>10)	0.028 \pm 0.01	0.008 \pm 0.004	0.145 \pm 0.06	0.15 \pm 0.06
P > 0.05				

Levels of chromium, cadmium, zinc and lead were slightly higher in adult sheep aged 2-4 years and in older sheep aged >4 years than in the younger group aged <2 years (Table 6). However, there was no statistically significant difference (P > 0.05) between the groups.

Table 6. Concentration of heavy metals in sheep according to age (n=14)

Age category (year)	Heavy metals concentration (Mean \pm SD) in mg/L			
	Cr	Cd	Pb	Zn
Young (<2)	0.022 \pm 0.006	0.005 \pm 0.002	0.134 \pm 0.008	0.134 \pm 0.007
Adult (2 - 4)	0.0276 \pm 0.007	0.007 \pm 0.003	0.1363 \pm 0.007	0.1363 \pm 0.008
Older (>4)	0.0275 \pm 0.0076	0.007 \pm 0.0028	0.1362 \pm 0.0067	0.1362 \pm 0.007
P > 0.05				

The mean concentration (mg/L) of chromium, cadmium and zinc and lead in cattle exposed to a heavy metal contaminated environment for > 4 years or 2-4 years was significantly ($P < 0.05$) higher than in cattle have been exposed for < 2 years (table 7).

Table 7. Concentration of heavy metals in cattle according to duration of exposure

Exposure duration	Heavy metals concentration (mg/L) (Mean \pm SD)			
	Cr	Cd	Pb	Zn
< 2 years	0.023 \pm 0.008	0.0062 \pm 0.004	0.103 \pm 0.07	1.31 \pm 0.159
2-4 years	0.041 \pm 0.009	0.008 \pm 0.003	0.144 \pm 0.05	1.30 \pm 0.217
> 4 years	0.092 \pm 0.007	0.009 \pm 0.003	0.18 \pm 0.032	1.33 \pm 0.186
P value	0.0015	0.0066	0.0162	0.8581

The mean concentration (mg/L) of Cr, Cd, Pb and Zn in sheep exposed to a heavy metal contaminated environment for > 2 years or 1–2 years were showed significantly ($P < 0.05$) higher value than sheep exposed for < 1 year (table 8).

Table 8. Concentration of heavy metals in sheep according to duration of exposure

Exposure duration	Heavy metals concentration (mg/L) (Mean \pm SD)			
	Cr	Cd	Pb	Zn
< 1 years	0.015 \pm 0.01	0.0034 \pm 0.002	0.055 \pm 0.06	1.175 \pm 0.05
1-2 years	0.026 \pm 0.002	0.01 \pm 0.0013	0.1361 \pm 0.09	1.243 \pm 0.12
> 2 years	0.058 \pm 0.004	0.08 \pm 0.002	0.1463 \pm 0.098	1.38 \pm 0.23
P value	0.0290	0.046	0.0151	0.0431

The mean values of Cr, Cd, Pb and Zn were higher in females than in males. However, there was no statistical significance ($P > 0.05$) of sex for heavy metal concentration (Table 9).

Table 9. Concentration of heavy metals in sheep and cattle according to sex

Sex	Heavy metals concentration (mg/L) (Mean \pm SD)			
	Cr	Cd	Pb	Zn
Female	0.0234 \pm 0.009	0.007 \pm 0.0035	0.1409 \pm 0.051	1.323 \pm 0.182
Male	0.0213 \pm 0.006	0.006 \pm 0.0034	0.132 \pm 0.0494	1.301 \pm 0.209
P value	0.2122	0.2029	0.5509	0.3613

The result showed that the mean of Cd and Zn in cattle was significantly ($P < 0.05$) higher than in sheep and the mean values of Cr and Pb in females were higher than in males, but not statistically significant difference ($P > 0, 05$) (table 10).

Table 10. Concentration of heavy metals according to species difference.

Species	Heavy metals concentration (mg/L) (Mean \pm SD)			
	Cr	Cd	Pb	Zn
Bovine	0.027 \pm 0.011	0.008 \pm 0.0041	0.14 \pm 0.062	1.36 \pm 0.2
Ovine	0.023 \pm 0.006	0.005 \pm 0.0018	0.135 \pm 0.006	1.23 \pm 0.11
P value	0.2428	0.0433	0.8416	0.0446

4. DISCUSSION

Water quality is considered an important nutrient component for livestock (Umar *et al.*, 2014). However, the main cause of heavy metal poisoning in animals is contaminated water. In the downstream region of the Little Akaki River, livestock farming largely relies on the river's water resources. As a result, significant amounts of heavy metals can be transferred to animals directly or indirectly from polluted water sources and subsequently enter the food chain and pose the greatest health risk to individuals consuming contaminated animal products (Umar *et al.*, 2014; Gupta *et al.*, 2021). Heavy metals are classified as one of the most serious forms of pollutants due to their significant toxicity, abundance and ability to accumulate in various animals (Bahiru and Teju, 2019) and plants (Amoah, 2011; Woldetsadik *et al.*, 2018).

Physical water quality parameters include salinity, turbidity, turbidity coefficient, pH and oxygen content. The mean temperature of the LAR water samples in the current study was 16.7-23.2 °C. The sampling point (S8) experienced the highest temperature. While the sampling point (S10) recorded the lowest temperature. The temperatures in (S8) were higher due to the following factors: Industrial effluents from textile, tanning, dye, plastic, rubber, and boiler production enter the river, which can discharge warm water into the river system. Compared to the results of the current research, almost similar results were obtained from (Deshu *et al.*, 2021) and (Benito, 2016) in the Little Akaki River (19.6 – 21 °C) and in the Kebena River (17.21 °C), respectively. However, a higher value (28.70-31.1 °C) was recorded for the Garra River in India by (Khan *et al.*, 2016). The temperature has a direct impact on the quality of the water, either by changing its taste and acceptability by the animals or by interfering with the bacteria in their gastrointestinal tract (Arias and Mader 2011).

The average pH values in this study were between 6.91 and 8.48. According to studies by Bakan *et al.*, (2010) and Khan *et al.*, (2016), untreated wastewater from industries such as tanneries, textile mills and rubber, which may contain detergents and soap, is one of the possible causes of the highest value at the sampling point (S8), indicating slightly alkaline river water. The area with the lowest pH was (S2), which can be attributed to the comparatively low pollution levels of the areas. The result of this study was comparable to the result reported by (Khan *et al.*, 2016) for the Garra River (7.10 - 8.30) in India. Water for animals should have a

pH between 6 and 8 (Hersom, 2008). Therefore, the results indicate that the pH of the water samples was within the acceptable ranges (6.5-8.5) for drinking water set by the Federal Ministry of Water Resources (FMoWR) in 2001 and by the World Health Organization (WHO) in 2001.

The average TDS and salinity of the water samples ranged from 42 - 642 and 46 - 139, respectively. The highest TDS and salinity levels could be caused by dissolved and suspended particles that people discharge into rivers as a result of their activities. These include agrochemicals, domestic wastewater and effluent from slaughterhouses in the city of Addis Ababa (Melaku *et al.*, 2007). The TDS level found in this investigation was remarkably close to the value (Benito, 2016) reported for the Kebena River in Addis Abeba, Ethiopia (40.43 - 640 mg/L). However it exceeded the figure (43 - 263 mg/L) for the Garra River in India that was reported by (Khan *et al.*, 2016). Excessive TDS concentration might influence the taste and palatability of water and lead to less than ideal animal development and production (Bharti *et al.*, 2017b; Giri *et al.* 2017; Kalia *et al.* 2017). High salt in mammals' drinking water can have a variety of acute symptoms, including excessive salivation, vomiting, diarrhea, blindness, convulsions, ataxia, confusion, and even paralysis (Umar *et al.*, 2014). Reduced intake of water and feed as well as slower weight increase has been documented as sub-chronic effects of high salinity in mammals' drinking water (Weeth and Haverland 1961).

In this study, the increased heavy metal content found at different sampling sites could be due to the amount of metal inputs from different sources. The average value of Cr was between 0.015 and 0.05 mg/L; the highest and lowest values were determined at sampling points (S4) and (S5), respectively. The increased Cr concentration is due to untreated wastewater from the tanning industry. Untreated wastewater from the tanning industry could be the cause of the increased Cr concentration. The Cr concentration in this study was higher than the reported value (0.01–0.04 mg/L) for the Jakara River in Malaysia (Mustapha *et al.*, 2013). However, the chromium content in this study for Little Akaki River was lower than in the previous report ($0.012 \pm 0.007 - 0.203 \pm 0.199$ mg/l) (Deshu *et al.*, 2021). At all sampling points, Cr levels did not exceed the allowable drinking water limit (0.05 mg/L) for drinking water set by the WHO (2011), FMoWR (2001) and the Northern Ireland Environmental Agency (NIEA) (2014).

The average Cd values at all sampling points were between 0.003 and 0.009 mg/L. The maximum and minimum values were determined at the sampling points (S9, S5) and (S1). According to Wuana and Okieimen (2011), cadmium typically comes from nickel-cadmium batteries, the cadmium coating of objects and cars, pigments, stabilizers, alloys, phosphate fertilizers and detergents. The level of Cd in all sampling sites were comparable with the previous value reported for the little akaki river by (Deshu *et al.*, 2021). However, the Cd concentration in water samples from the LAR River was lower than the reported value (0.01–1.00 mg/L) for the Jakara River in Malaysia. At most sampling points the cadmium content exceeded the upper permissible limits (0.003 mg/L) of the FMoWR (2001). Therefore, due to its toxicity, bio-accumulative properties and potential impact on farm animal health and human food safety, care must be taken to ensure the concentration of cadmium exceeds recommended levels.

The Pb concentration varied on average between 0.09 and 0.31 mg/L at the sampling sites, with sampling site (S9) and site (S5) recording the highest values. The average lead level in this study was higher than that for Jakarta River (0.01-0.04 mg/L) by (Mustapha *et al.*, 2013) and for LAR (0.031-0.12 mg/L) by (Deshu *et al.*, 2021) specified value). Pb levels in the water samples were above the recommended levels for bovine drinking water established by the Canadian Council of Ministers of the Environment (CCME) (2005) (0-0.01 mg/L), the Department of Water Affairs and Forestry (DWAFF) (0.015 mg/L) and the National Academy of Sciences (NAS) (0.015 mg/L). Lead is a hazardous metal that can have a negative impact on an animal's reproductive hormones (Valente-Campos *et al.*, 2014). Therefore, the Pb content exceeding the guideline value deserves special attention due to its toxic effect, bioaccumulation as well as the negative impact it can have on the food safety of animals and humans.

In the current study, mean zinc levels at sampling sites ranged from 0.029 to 0.94 mg/L, with peak concentrations recorded at (S10) and (S8), respectively. The highest Zn value at the sampling site (S10) was obtained due to the inflow of industrial effluents in the upper reaches and the extensive use of agricultural chemicals such as fertilizers and pesticides (Boateng *et al.*, 2015; Wuana and Okieimen 2011). The Zn concentration in LAR water was higher than the

value reported by (Abdel-Satar *et al.*, 2017) (0.01–0.115 mg/l) for the Nile in Egypt and by (Oketola *et al.*, 2013) (0.01–0.07 mg /l) for the Ogun River in Nigeria.

In general, the variations in heavy metal concentrations in national and internal river water could be due to different sources and amounts of environmental inputs

The possible sources of heavy metals in livestock were known; Heavy metals enter the body of animals through consumption of polluted water and feed; it was observed that the animals had direct access to the river water for drinking. Therefore, water is one of the most important sources of heavy metal poisoning in livestock (Mukesh *et al.*, 2008). Consumption of heavy metal residues in the water, soil and forage produced in the area across the river could clearly play a role in the increased concentration of heavy metals in the blood (Reglero *et al.*, 2008; Rajaganapathy *et al.*, 2011).

During the dry season, animals reared near the LAR completely depend on the river water for drinking. However, animals that are far from river water get their water from other sources such as groundwater or other rivers. Therefore, this study included animals from three villages based on their proximity to the river; Village-1 ('Qersa') and Village-2 ('Ichu') are located 500 m - 1 km from the river and Village-3 (Gelaan-Gudda) are located > 2 km from the river. The result of the current study showed that animal populations reared in polluted areas are exposed to dangerous toxic heavy metals such as Cr, Cd, Pb and Zn, which can accumulate in higher concentrations in the blood. Although no evidence of animal toxicity was observed in this study, the possibility of chronic effects due to the higher concentration of Cr, Cd, Pb and Zn cannot be excluded. The sample was collected from the animals that were apparently healthy and showed no clinical signs of heavy metal poisoning. However, some animals had skin problems such as rough hair, poor body condition, and could not get pregnant easily.

The mean concentration level of Cd in animals of village-1 and village-2 were 0.009 ± 0.003 mg/l and 0.0083 ± 0.0027 respectively, which was statistically significant ($p < 0.05$) and higher than the obtained level 0.002 ± 0.0006 mg/l for animals of Village-3 (table 2). The mean concentration of Cd in the animals from village-1 and village-2 was 0.009-0.003 mg/l and 0.0083-0.0027, respectively, which was statistically significant ($p < 0.05$) and higher than the concentration obtained from village-3 (0.002-0.0006 mg/l). (Table 2). Therefore, the Cd level in

the study areas (Village-1 and Village-2) was above the permissible limit values for cadmium in the blood of animals (0.007 mg/l) (Crouse *et al.*, 1983). Cadmium has been reported to reduce intestinal zinc absorption and hepatic zinc reserves in cattle as it competes for the binding site of certain proteins such as MT (Goyer 1997; McDowell, 2003). This could be a possible reason for high concentrations. The Cd content in Village-1 and Village-2 is higher than in Village-3 and the Zn content in Village-1 and Village-2 is lower than in Village-3. Therefore, with regard to the current work, most of the animals in the study areas (Village-1 and Village-2) drink water from the river and grazing in this area is not safe for human consumption.

Lead is a heavy metal that poses major occupational and environmental concerns. As an environmental pollutant, Pb is often associated with Cd as both elements have similar properties and their health effects are additive (Tella *et al.*, 2012). The mean blood lead levels in Village-1 and Village-2 were in the range of 0.16 ± 0.033 mg/l and 0.154 ± 0.023 mg/l, respectively, and were thus significantly ($p < 0.05$) above the observed value (0.013 ± 0.001 mg/l) for blood from Village-3 (Table 2). Therefore, the Pb levels determined in this study were comparable to those of other researchers (Abdou *et al.*, 2012; Rodriguez *et al.*, 2015) who also reported higher blood Pb levels in different animals. However, the lead concentration obtained was above 0.02 mg/l, which is an allowable limit for trace metals in the blood of animals (Ogabiela *et al.*, 2011; Gupta *et al.*, 2021). In general, high lead levels are considered to be a feature of chronic lead exposure (Olawoyin *et al.*, 2018). This implies that animals from the studied areas, especially from Village-1 and Village-2, are unfit for consumption and could pose a health hazard to humans via the food chain.

Although chromium and other microelements are necessary to maintain the body metabolic processes, they can be toxic in larger amounts. Chromium and other microelements are essential for maintaining the body's metabolic system, but in higher concentrations they can cause poisoning. The bioavailability and cell membrane penetrating potential of this metal determine its toxicity (Cuberos, 2009). In this study, the mean value of Cr in village-1 and village-2 animals was 0.031 ± 0.007 and 0.0305 ± 0.005 , respectively, which is statistically significant ($p < 0.05$) and higher than the value (0.009 ± 0.002) in village-3. The chromium concentrations required for livestock are between 0.3 and 1.6 mg/kg or 0.05 mg/l; higher levels

than these levels are harmful to animals and adversely affect ruminant reproductive performance (McDowell, 2003; CCME, 2005; Oklowski 2009).

Experimental research has shown that exposure to heavy metals, particularly Cd and Pb, at concentrations above the allowed limit leads to a significant change in biochemical and hematological markers such as AST, ALP, BUN, total protein, albumin, total leukocyte count, differential leukocyte count, PCV and Hemoglobin concentration (Swarup *et al.*, 2007; Nisha *et al.*, (2009; Zaki *et al.*, 2010; Patra., 2011; Sellaoui *et al.*, 2016 and Milena *et al.*, 2019).

In this study, the results of hematological analysis of WBC, PCV and Hb in the blood sample from the study areas (Village-1 and Village-2) showed a normal range, but compared to Village-3, these parameters were fewer in number. The results of this research were consistent with other studies conducted using a variety of animal models, routes of exposure and doses. Heavy metals have been found to have a reducing effect on the amount of PCV, HB and WBC (Zaki *et al.*, 2010; Sharma *et al.*, 2011; Sellaoui *et al.*, 2016; Mladenovic *et al.*, 2019). The higher neutrophil counts in this study were consistent with the results of two different reviews by (Mugahi *et al.*, 2003; Farkhondeh *et al.*, 2014), but contradicted the results of (Muhammad *et al.*, 2018); In contrast to this study, lower neutrophil counts were found. Therefore, uptake of heavy metals such as Cd and Pb by ruminants from the natural environment is a possible explanation for the differences in hematological tests observed between the three villages in our study.

In this study, a significantly higher activity of AST and ALP was found in the blood of Village-1 and Village-2 ($P < 0.05$) than in Village-3; while values of other biochemical parameters such as BUN, total protein and albumin were statically significant ($P < 0.05$) and decreased in the blood samples from Village-1 and Village-2 compared to the blood sample from Village 3. The observed biochemical parameter; The decreased total protein and albumin levels and increased AST and ALP activity in this study were consistent with research by (Swarup *et al.*, (2007); Khalid *et al.*, 2021) who also reported their findings on altered liver and renal function parameters reported in cattle exposed to environmental heavy metal exposure.

The current result of a significantly higher AST activity and reduced total protein and albumin levels clearly shows that the liver problems in cattle and sheep occur with higher blood lead and cadmium levels. Membrane damage to vital organs such as the heart, liver, and kidney results in a two to four-fold increase in AST. Thus, enzyme levels above normal indicate some level of tissue or cellular dysfunction. Slight changes in the kidney lead to a variation in the BUN concentration (Godt *et al.*, 2006).

5. CONCLUSION AND RECOMMENDATION

Various natural and man-made activities are responsible for river water degradation; the quality of the Little Akaki River has been primarily affected by man-made activities such as unregulated and inappropriate discharges of waste products from industry, households, municipalities, sewers, agrochemicals, etc. Consequently, physico-chemical properties such as temperature, TDS, turbidity and salinity exceeded Ethiopia's Recommended Drinking Water Quality Standard (FMoWR) and Guideline Limit. Moreover, the introduction of hazardous substances such as toxic heavy metals especially Lead and Cadmium were exceeded the permissible limit values set for livestock drinking. However during the dry season, animals reared in the lower courses of the little akaki river were highly affected from direct or indirect exposure to the river water. Thus, the result of this study showed that heavy metals, especially cadmium and lead, were transferred to the animals in small doses from the contaminated environment (feed and water) and accumulated in the blood without showing any clinical manifestations. As a result, hematological and biochemical parameters changed, this could be an indication of cell damage due to heavy metal toxicity.

Based on aforementioned conclusion the followings were recommended:

- ✚ Additional research is needed in the study region of blood and water samples, water and blood samples for heavy metals such as arsenic, mercury, manganese, and iron, which were not addressed in this study.
- ✚ Proper monitoring and control of waste disposal should be applied by the concerned body
- ✚ Organic farming should be encouraged and the use of agricultural pesticides, herbicides and phosphorus fertilizers minimized or avoided
- ✚ Spring water for livestock should be free of heavy metals
- ✚ Irrigation water sources for pastures and crops must be free of heavy metals
- ✚ Anthropogenic sources of water and pasture contamination should be minimized or avoided
- ✚ Feed and water containing heavy metals should not be given to the animals
- ✚ Plastic pipes instead of iron steel pipes should be used

- ✚ All sources of heavy metals such as batteries, cement, paint, lubricating oils, etc. should be kept away from animal feed and water
- ✚ Continuous monitoring and evaluation of environment and animal tissues should be implemented

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