

ORIGINAL ARTICLE

Coexistence of diverse heavy metal pollution magnitudes: Health risk assessment of affected cattle and human population in some rural regions, Qena, Egypt

Hassan M. Diab¹, Mohammed A. Alkahtani², Ahmed S. Ahmed³, Atef M. Khalil⁴, Mohmmmed A. Alshehri², Mohamed A. A. Ahmed⁵, Ibrahim F. Rehan⁶, Ahmed A. Elmansi², Ahmed E. Ahmed^{2,7}

¹Department of Animal and Poultry Health and Environment, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

²Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia

³Department of Food Hygiene and Control (Milk Hygiene), Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

⁴Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

⁵Central Laboratory of Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

⁶Department of Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Menofia University, Shebin Alkom, Menofia, Egypt

⁷Department of Theriogenology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

ABSTRACT

Objective: The purpose of this study was to measure the mean concentrations of heavy metals including aluminum (Al), arsenic, nickel (Ni), mercury, lead (Pb), and cadmium (Cd) and to assess the health hazards due to the exposure of cattle/human population to a distinct or the mixture of heavy metals through various sources.

Materials and methods: A total of 180 samples including water sources, animal feed, and raw cows' milk from rural regions in Qena, Egypt, were examined using the inductively coupled plasma emission spectrometer (ICP; iCAP 6200).

Results: The data highlighted heavy metal pollution with variable concentrations among most of the investigated regions. All concentrations of Al, Ni, and Cd detected in the feeding stuff showed a strong correlation to their respective levels in milk rather than those detected in water ($R^2=0.072$ vs. 0.039 , 0.13 vs. 0.10 , and 0.46 vs. 0.014 , respectively) ($p < 0.05$). Anisocytosis and poikilocytosis with a tendency to rouleaux formation were evident, and basophilic stippling was a pathognomic indicator for heavy metal toxicity, especially Pb. Leukopenia and macrocytic anemia were shown in 50% and 65% of examined cattle, respectively. The target hazard quotients values were more than one (>1) for all heavy metals from water intake for both children and adults and Al and Cd in milk for children, and the hazard index values were indicated higher for noncarcinogenic health hazards. The target cancer risk values predispose people in the surveyed villages to higher cancerous risks due to exposures to the mixture of heavy metal through the consumption of water and milk.

Conclusion: The bioaccumulation and transmission of heavy metal mixtures from water sources and feeding material have detrimental influences on milk pollution and cattle health which seem to be a serious issue affecting public health in those rural communities.

ARTICLE HISTORY

Received January 05, 2020

Revised March 21, 2020

Accepted March 24, 2020

Published May 10, 2020

KEYWORDS

Heavy metals; health hazards; hematological markers; polluted water and milk



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Introduction

The ever-increasing urbanization and industrial revolution in Egypt have been led to heavy metal contamination of surface water and agricultural sectors, which, in turn,

bioaccumulated and transmitted through the food chain [1]. Heavy metals are critical pollutants due to their widespread implementation in various anthropogenic activities as well as their resistance in the environment and potential

Correspondence Hassan Mahmoud Diab ✉ hassan.abdelatef@vet.svu.edu.eg Department of Animal and Poultry Health and Environment, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

How to cite: Diab HM, Alkahtani MA, Ahmed AS, Khalil AM, Alshehri MA, Ahmed MAA, Rehan IF, Elmansi AA, Ahmed AE. Coexistence of diverse heavy metal pollution magnitudes: Health risk assessment of affected cattle and human population in some rural regions, Qena, Egypt. *J Adv Vet Anim Res* 2020; 7(2):345–359.

toxic risks on various organisms [2]. Polluted water and feedstuff are risky factors directly affecting animal health and indirectly human health [3]. Heavy metals are potential bioaccumulative toxicants causing serious health hazards via the drinking water and animal feeds [4]. Heavy metals get their pathway to cattle milk from various sources [5], and accordingly, the intake of polluted milk negatively affects the public health of human. On the other hand, the hematological changes and biochemical profiles of dairy cows in response to heavy metals are valuable indicators for liver and kidney disorders, which reflect the general health state [6]. Chronic exposure to metal toxicity leads to anemia, teratogenic effects, hematotoxicity, and cancer [7].

Egypt is ranked among countries that have a lower middle income according to the indicators of the World Bank Development. Egypt's economy is one of the largest and most diversified in the Middle East, which focused on industrial and agricultural sectors. Rapid economic growth and increasing population are the leading factors, which contribute to the release of higher levels of various environmental pollutants including heavy metals, especially in rural areas, despite attempts for the implementation of counteract strategy to mitigate such pollution. A continuous measuring and estimation of heavy metal concentration in water, milk, and animal feedstuff in Egypt are carried out, and there are few available data on the ecology and dietary intake of heavy metal levels among the Egyptian population [8]. Therefore, it is imperative to evaluate and quantify the heavy metal contamination as well as to understand their spatial distributions in rural environments. The current work aims to investigate the environmental contamination with heavy metals and clarify the hematological and biochemical profile response in the dairy cattle. Heavy metals are determined accurately by inductivity coupled plasma optical emission spectrometry as a widely used method measuring the heavy metals in food and environmental samples [9].

It is essential to determine the occurrences and monitor the levels of heavy metal conjugated with health hazard assessments seeking to evaluate the exposure of populace to certain ecological pollutants through various exposure sources simultaneously. The estimation of heavy metal intake in the environmental sources such as water, feeding material, and raw cows' milk is evaluated and compared to the maximum permissible limits (MPL), acceptable daily intake (ADI), and maximum tolerable dietary level (MTDL) of those metals for both human and animal in those rural regions in Qena, Egypt.

Materials and Methods

Ethical approval

Ethical approval was granted (approval number: Directive 2018/13/VET-SVU) by the Research Ethics Committee of

the Faculty of Veterinary Medicine, South Valley University, Egypt. All procedures involving animals during the current study were performed in accordance to the institutional guideline which follows the international guidelines of the National Institutes of Health, United States, and World Organization for Animal Health (OIE).

Experimental design

The study assessed the concentrations of various toxic metals including aluminum (Al), arsenic (As), nickel (Ni), mercury (Hg), lead (Pb), and cadmium (Cd) in water samples obtained from sources used for drinking of human, animals, and other domestic chores, in addition to the cow's feeding material. The residual concentrations of those metals were detected in the raw cow's milk, and finally, the blood samples from the same animals were collected for studying the hematological and biochemical changes in response to those metals. The study was performed in variable geographical area targets of animal household rearing in four villages around Qena Province, South Egypt.

Water and feeding stuff

A total of 120 samples of drinking water and feeding material (60 for each category) were collected. The water samples were collected in Falcon™ tubes according to standard methods [10]. The samples were collected from the dry corn rations locally formulated by the farmers in such localities and used as feeding material for calves and cows for analysis [11]. The feeding samples were collected in clean sterile plastic bags. All water and feeding material samples were transferred to the laboratory.

Cow's milk and blood sampling

A total of 60 adult milking nonpregnant pluriparous cows, as 15 animals from each village, were used. From each cow, about 50 ml of raw liquid milk samples were collected in clean sterilized Falcon™ 50-ml conical centrifuge tubes. The milk samples were kept in ice packs and stored in the laboratory at -20°C until used for heavy metal assays. About 5 ml of blood were collected from each cow into sterile gel glass tubes. The blood samples were divided into two portions: whole blood film staining with Giemsa was obtained for complete blood cell count (CBC) and the other portion was allowed to coagulate to obtain the serum for biochemical analysis. The blood films were immediately performed after collection. The other portion of blood samples were immediately transported for sera harvesting within an hour by 15-min centrifugation at 3,000 rpm and then stored in plastic tubes at -20°C till used for assaying the liver/kidney function markers and C-reactive protein measurement. All milk and blood samples were collected per a week interval.

Heavy metal assays

The levels of Al, As, Ni, Hg, Pb, and Cd in water, feeding material, and milk specimens were measured through inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6200) at the Central Laboratory for Chemical Analysis, Faculty of Agriculture, Assiut University, Egypt. Wet digestion was performed according to the method adopted by the previous study [12]. The samples were prepared for obtaining a solution by digestion. The leftover solution obtained from those digested samples was filtrated and diluted with deionized distilled water for measuring the mentioned metals by the spectrometer.

The obtained results were matched with the MPLs of the local Egyptian chemical standards, Egyptian Organization for Standardization and Quality Control, World Health Organization (WHO), and International Dairy Federation (IDF) latest guideline standards.

Assessment of daily and weekly intake

The calculations of the estimated daily intake (EDI) and estimated weekly intake (EWI) of heavy metals by the animal and human in the examined drinking water, feeding material, and milk samples were performed according to equations described before [9]. The results were compared with ADI and provisional tolerable weekly intake (PTWI) according to the standards provided by the International Organization for Standardization.

Target hazard quotient (THQ)

The magnitude relation of exposure to toxic heavy metal and, therefore, the reference dose that is the highest level, at which no adverse health effects are predicted, is referred to as THQ. The reference dose is restricted to the heavy metal being evaluated. The THQ measures the risk of the occurrence of noncarcinogenic health consequences due to toxicity with heavy metals. Noncarcinogenic health effects do not seem to be expected once the THQ is <1. However, there is an occasion that adverse health effects can be practiced if THQ is >1. The THQ was calculated according to the method of Antoine et al. [13] based on the following equation:

$$THQ = EFR \times Ed \times FIR \times CRFD \times BWa \times ATn \times 10^{-3}$$

Hazard index (HI)

The summation of the distinct THQ of heavy metals evaluated for every food category is named as HI. The HI considers that the intake of a distinct type of food might Pb to synchronized exposure to several toxic heavy metals [13]. If the HI is >1, there is a possibility for noncarcinogenic

health implications. The HI was calculated according to the following formula:

$$HI = \sum THQ_n$$

Target cancer risk (TCR)

The probable risk related to the exposure of oncogenic agents all over the lifespan exposure period was measured using the TCR [13]. If the TCR is higher than the maximum values of 10^{-6} – 10^{-4} [14], there are likely oncogenic health implications. The TCR was calculated according to the following equation:

$$THQ = EFR \times Ed \times FIR \times C \times CPSoBWa \times ATc \times 10^{-3}$$

A total TCR is calculated by adding the individual TCR data by the following equation:

$$Target\ Cancer\ risk_{total} = Target\ cancer\ risk_{As} + Ni + Pb + Cd$$

Table S1 shows detailed information regarding THQ, HI, and TCR formula.

Biochemical assays

Liver function markers

The concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in sera were evaluated as mentioned before [15]. Serum alkaline phosphatase (ALP) level was calorimetrically determined using Randox Kit as stated by Belfield et al. [16]. Total protein and bilirubin concentrations were calorimetrically measured by the biuret method [17].

Kidney function markers

The concentrations of creatinine in sera were measured according to Henry [18]. The levels of urea and uric acid were assayed using Urease–Berthelot reaction [19].

Statistical analysis

Mean \pm SEM was chosen to express all of the biochemical data. The statistical analyses were performed by using one-way analysis of variance followed by Newman Keuls as a *post hoc* test. The differences between the groups were considered to be significant at $p < 0.05$. The nonsignificant differences were confirmed by the Student's *t*-test. The significant differences between the values detected were compared to the absolute range values by using the one-sample *t*-test. Multiple correlation matrices and linear regression were performed to detect the correlation between different parameters. The Statistical Package for the Social Sciences and GraphPad Prism for statistical figures (San Diego, CA, version 5) were used to perform the statistical analyses.

Results

Heavy metal analysis

The incidence and levels of heavy metals in the drinking water, feeding material, and raw cow's milk are shown in Table 1A. Lead and Al were detected in most of the collected samples. Moreover, As was only detected in feeding material, whereas raw cow's milk proved to be free from Hg. The results also showed that Al has the highest level detected at a concentration of 9.88, 92.05, and 30.04 mg/l or kg from water, feeding material, and milk, respectively.

It was revealed that most of the analyzed water, feeding material, and milk sources did not meet the local/international regulations concerning their respective MPL, especially Pb and Al, as shown in Table 1B, which expose the influence of those toxicants on the health of both human and animal and trigger additional monitoring and investigation for the application of counteract measures.

Assessment health hazards due to the exposure of heavy metals

To assess the health hazard linked to heavy metal pollution of water and milk, ADI and PTWI have been compared

with EDI and EWI levels for children and adult human consumption as shown in Table 2A and B.

These results have shown that 100% ($n = 60$) of the EDI of milk examined for both Al and Pb in milk exceeded their respective ADI for both children (408.51 vs. 0.0036 and 2.11 vs. 0.0035 mg/kg b.w, respectively) and adult human (104.75 vs. 0.0036 and 0.54 vs. 0.0035 mg/kg b.w, respectively). On the other hand, 65% ($n = 39$) and 95% ($n = 57$) of the milk samples examined for Ni and Cd exceeded their respective ADI% for children (1.05 vs. 0.02 and 0.16 vs. 0.001 mg/kg b.w, respectively) and adult human (0.27 vs. 0.02 and 0.04 vs. 0.001 mg/kg b.w, respectively). The results of water samples examined for heavy metal pollution exceeding the ADI in both children and adult human were the same. They varied from 35%, 40%, 65%, 80%, and 100% more than the ADI% for Hg, Ni, Cd, Al, and Pb, respectively.

The estimated daily levels (EDLs) compared to the MTDL of heavy metals in milk, water, and feeding material samples used for calves' consumption are shown in Table 4. Milk used for calves' consumption showed variable EDL of heavy metal pollution exceeding the MTDL%, such as 35%, 55%, and 65% for Pb, Cd, and Al, respectively. However,

Table 1. Mean \pm S.E of Al, As, Ni, Hg, Pb, and Cd in the examined samples of drinking water, feeding material, and raw cow's milk are shown in this table.

(A)	Water (mg/l)			Feeding stuff (mg/kg)			Raw cow's milk (mg/l)		
	+ve Cases		Mean \pm S.E	+ve Cases		Mean \pm S.E	+ve Cases		Mean \pm S.E
	No.	%		No.	%		No.	%	
Al	48	80	9.88 \pm 0.92	54	90	92.05 \pm 10.0	60	100	30.04 \pm 6.37
As	0	0	0	51	85	0.152 \pm 0.04	0	0	0
Ni	24	40	0.43 \pm 0.08	42	70	1.550 \pm 0.20	39	65	0.09 \pm 0.03
Hg	24	40	0.37 \pm 0.16	9	15	0.079 \pm 0.03	0	0	0
Cd	39	65	0.05 \pm 0.02	60	100	0.260 \pm 0.08	57	95	0.01 \pm 0.003
Pb	60	100	0.77 \pm 0.07	60	100	0.410 \pm 0.05	60	100	0.18 \pm 0.010

(B)	Water			Feeding stuff			Raw cow's milk		
	MPL (ppm)	> MPL		MPL (ppm)	> MPL		MPL (ppm)	> MPL	
		No.	%		No.	%		No.	%
Al	0.200 ^a	45	75	0.2 ^s	54	90	0.02 ^m	60	100
As	0.010 ^b	0	00	4 ^h	0	00	0.14 ⁿ	0	0
Ni	0.020 ^c	24	40	50 ⁱ	0	00	0.2 ^o	6	10
Hg	0.001 ^d	21	35	0.1 ^j	9	15	0.5 ^p	0	0
Cd	0.003 ^e	30	50	0.1 ^k	18	30	0.026 ^q	9	15
Pb	0.010 ^f	48	80	0.2 ^l	39	65	0.049 ^r	60	100

The incidence of those metals exceeding their MPL is shown in Table 1B. All data are presented as mean \pm SE for a total of 60 samples examined per each category ($n = 60$). All standard references of values are shown below.

a = WHO (2003a) [50]; g = EOSQC7136/2010 [51]; m = EOSQC7136/2010 [51]; b = Codex Standard 193-1995 [52]; h = Codex Standard 193-1995 [52]; n = Codex Alimentarius (2013) [53]; c = WHO (2003b) [54]; i = Commission Regulation, (2013) [55]; o = Codex Alimentarius (2013) [53]; d = Codex Standard 193-1995 [52]; j = NRC (2005) [56]; p = Codex Alimentarius (2013) [53]; e = WHO (2006) [57]; k = Codex Standard 193-1995 [52]; q = IDF (1979) [58]; f = WHO (2006) [57]; l = European Commission (2003) [59]; r = IDF (1979) [58].

Table 2. Comparative analyses of ADI and PTWI to EDI and EWI of heavy metals in the drinking water and raw cow's milk, for children and adult human, are shown in 2A and B, respectively.

		Children						
(A)	Metals (mg/l)	Al	As	Ni	Hg	Pb	Cd	
	ADI	0.0036 ^a	0.015 ^b	0.02 ^c	0.005 ^d	0.0035 ^e	0.001 ^f	
	PTWI	0.0252	0.105	0.14	0.035	0.025	0.007	
Contribution%	Milk	48.4	0.00	5.2	0.000	5.9	6.3	
	Water	51.6	0.00	94.8	100	94.1	93.7	
EI	EDI	843.25	0.00	20.08	16.43	35.95	2.47	
	EWI	5,902.73	0.00	140.58	115.04	251.63	17.26	
Milk	DI	EDI	408.51	0.00	1.05	0.000	2.11	0.16
		>ADI %	100%	0.00	39 (65%)	0.000	100%	57 (95%)
	WI	EWI	2,859.57	0.00	7.37	0.000	14.74	1.09
>PTWI %		100%	0.00	65	0.000	100 %	57 (95%)	
Water	DI	EDI	434.74	0.00	19.03	16.43	33.84	2.3
		>ADI %	48 (80%)	0.00	24 (40%)	21 (35%)	100%	39 (65%)
	WI	EWI	3,043.16	0.00	133.21	115.04	236.88	16.17
>PTWI %		48 (80%)	0.00	24 (40%)	21 (35%)	100%	39 (65%)	
		Adult human						
(B)	Metals (mg/l)	Al	As	Ni	Hg	Pb	Cd	
	ADI	0.0036 ^a	0.015 ^b	0.02 ^c	0.005 ^d	0.0035 ^e	0.001 ^f	
	PTWI	0.0252	0.105	0.14	0.035	0.025	0.007	
Contribution %	Milk	18.7	0.0	1.3	0.000	1.5	1.7	
	Water	81.3	0.0	98.7	100	98.5	98.3	
EI	EDI	560.77	0.0	20.2	17.24	36.04	2.47	
	EWI	3,925.35	0.0	141.62	120.67	252.26	17.26	
Milk	DI	EDI	104.75	0.0	0.27	0.000	0.54	0.04
		>ADI %	100%	0.0	39 (65%)	0.000	100%	57 (95%)
	WI	EWI	733.23	0.0	1.89	0.000	3.78	0.28
>PTWI %		100%	0.0	39 (65%)	5	100%	57 (95%)	
Water	DI	EDI	456.02	0.0	19.97	17.24	35.5	2.43
		>ADI %	48 (80%)	0.0	24 (40%)	21 (35%)	100%	39 (65%)
	WI	EWI	3,192.13	0.0	139.73	120.67	248.48	16.97
>PTWI %		48 (80%)	0.0	24 (40%)	21 (35%)	100%	39 (65%)	

All standard references of values are shown below.

a = Codex Alimentarius (2013) [53]; b = JECFA (1982; 1989; 2000) [60]; c = US-EPA (2005) [61]; d = JECFA (1982; 1989; 2000) [60]; e = JECFA (1982; 1989; 2000) [60]; f = Codex Alimentarius (2013) [53].

EDL values of As, Ni, and Hg did not exceed the MTDL in the same samples. Water samples showed EDL of 0%, 35%, 40%, 55%, 75%, and 80% exceeding MTDL approved to As, Hg, Ni, Cd, Al, and Pb, respectively. Furthermore, feeding material showed variable EDL exceeding their respective MTDL as 5% for As, 15% for Hg and Pb, 55% for Ni, 70% for Al, and 95% for Cd.

The comparison of EDL and MTDL of heavy metals in raw cow's milk, water, and feeding material samples used

for growing and lactating cattle consumption is shown in Table 5A and 5B, respectively. The EDL of heavy metals exceeding their respective MTDL showed variable percentages from low to medium and high levels in both the growing and lactating cattle. The EDL of heavy metal pollution in the drinking water used for both the growing and lactating cattle exceeded the MTDL by 30%–40% for Hg and Ni, 50% for Cd, and 75% for Al and Pb. Furthermore, those EDLs of heavy metals in the feeding material consumed

Table 3. THQ, HI, TCR, and total target cancer risk for Al, As, Ni, Hg, Pb, and Cd analyzed in drinking water and raw cow's milk, for children and adult human.

		Children						
(A)	THQ	Al	As	Ni	Hg	Pb	Cd	HI= \sum THQ
	Milk	3.57E+00	0	4.61E-01	0	5.12E-01	1.36E+00	5.91E+00
	Water	3.80E+00	0	8.33E+00	4.79E+02	8.23E+00	4.04E+01	5.40E+02
		Adult human						
(B)	THQ	Al	As	Ni	Hg	Pb	Cd	HI= \sum THQ
	Milk	2.44E-01	0	3.15E-02	0	3.50E-02	9.31E-02	4.04E-01
	Water	1.06E+00	0	2.33E+00	1.34E+02	2.30E+00	1.13E+01	1.51E+02
		Adult human						
(C)	TCR	Ni	Pb	Cd	Target cancer risk _{TOTAL} = \sum TCR			
	Milk	4.59E-04	4.59E-06	1.516E-05	4.79E-04			
	Water	3.39E-02	3.02E-04	9.21E-04	3.52E-02			

If the THQ is >1, then there is a possibility that adverse health effects could be experienced. Furthermore, if the HI is >1, there is the potential for adverse noncarcinogenic health effects. If the TCR is higher than the maximum values of 10^{-6} – 10^4 , there is the potential for adverse carcinogenic health effects.

Table 4. Comparative analysis of EDL compared to MTDL (mg/kg b.w.) of heavy metals in raw cow's milk, water, and feeding material samples for calves.

Metals	TDL	Milk		Water		Feed		Total EDL	Contribution (%)		
		EDL		EDL		EDL			Water	Milk	Feed
		MTDL	EDL	>MTDL (%)	EDL	>MTDL (%)	EDL				
Al	1,000 ^a	4,713.6	65	3,800.2	75	3,398.58	70	11,912.3	39.6	31.9	28.5
As	50 ^b	0	0	0	0	5.62	5	5.61	0	0	100
Ni	50 ^c	12.15	0	166.35	40	57.05	55	235.54	70.6	5.2	24.2
Hg	2 ^d	0	0	143.66	35	2.91	15	262.87	54.6	44.3	1.1
Pb	30 ^e	24.3	35	295.81	80	15.3	15	335.41	88.2	7.2	4.6
Cd	0.5 ^f	1.79	55	20.2	55	9.77	95	31.76	63.6	5.7	30.7

a – f = NRC, (1980) [62].

by the growing and lactating cattle, either, exceeded the MTDL by 10%–15% for Hg and Pb, 55%–65% for Ni and Al, and 95% for Cd.

The THQ data of Al, As, Ni, Hg, Pb, and Cd by individual intake from water and milk (Table 3) showed significantly higher values for children than adult. Besides, THQ values were >1 for all heavy metals from water intake for both children and adults. On the contrary, THQ value for elements from milk consumption showed the values in decreasing order Al > Cd > Ni > Pb with THQ of Al and Cd, which were >1 for children. Moreover, the results of HI value were far above 1 for both children (5.40E+02) and adults (1.51E+02). Besides, the effect of the mixture of metals in cow's milk produced in those areas showed HI value >1 for children (5.91E+00) but not for adult which had HI value <1. The oncogenic hazard due to drinking of water in the studied villages depends on the separable effects of Ni, Pb, and

Cd (Table 3), indicating that Ni possessed the highest risk than Pb and the lowest than Cd, whereas, for milk, the risk was in decreasing order for Ni > Cd > Pb. The analyses of the sum of the cancer risk indexes signified by the heavy metals as a group were in water (3.52E-02) and milk (4.79E-04).

Hematological and biochemical findings

The effects of heavy metal exposure on some hematological and biochemical parameters in cattle including the CBC and liver and kidney function tests are shown in Table 6. Examined blood films showed anisocytosis, marked by microcytes and macrocytes, and poikilocytosis, marked by keratocytes and elongated cells. Furthermore, there was a tendency to rouleaux formation, and the reticulocytes were observed. Interestingly, some basophilic stippling cells were observed as pathognomic evidence to heavy metal toxicity, especially Pb toxicity, as shown in Figure 3.

Table 5. Comparative analysis of the EDL to MTDL % of heavy metals from examined water and feeding material samples for growing and lactating cattle is shown in 5A and 5B, respectively.

(A)	TDL	Water			Feeding material		Total EDL	Contribution (%)	
		EDL			EDL			Water	Feed
		MTDL	EDL	>MTDL %	EDL	>MTDL %			
Al	1,000 ^a	1.954.36	45 (75%)	3,034.45	39 (65%)	4,988.81	39.2	60.8	
As	50 ^b	0	0	5.01	0	5.01	0	100	
Ni	50 ^c	85.55	24 (40%)	50.93	33 (55%)	136.48	62.7	37.3	
Hg	2 ^d	73.89	18 (30%)	2.6	9 (15%)	76.48	96.6	3.4	
Pb	30 ^e	152.13	45 (75%)	13.66	9 (15%)	165.79	91.8	8.2	
Cd	0.5 ^f	10.39	30 (50%)	8.72	57 (95%)	19.11	54.4	45.6	

(B)	TDL	Water			Feeding material		Total EDL	Contribution (%)	
		EDL			EDL			Water	Feed
		MTDL	EDL	>MTDL %	EDL	>MTDL %			
Al	1,000 ^a	1,956.75	45 (75%)	2,700.59	39 (65%)	4,656.34	42.0	58.0	
As	50 ^b	0	0 (0%)	4.46	0 (0%)	4.46	0	100	
Ni	50 ^c	85.65	24 (40%)	45.33	24 (40%)	130.98	65.4	34.6	
Hg	2 ^d	73.97	18 (30%)	2.31	9 (15%)	76.28	97	3	
Pb	30 ^e	152.32	45 (75%)	12.16	6 (10%)	164.47	92.6	7.4	
Cd	0.5 ^f	10.4	30 (50%)	7.76	57 (95%)	18.16	57.3	42.7	

a – f = NRC, (1980) [62].

Table 6. Effects of heavy metal exposure on some hematological and biochemical parameters in cattle.

Parameters	Conventional (USA) units	Min	Max	Mean ± SE	SI units	Ref range	Absolute range
Hematology	RBCs	3.5	6.5	4.84 ± 0.13 ^{NS}	×10 ⁶ /μl	5–10	5
	WBCs	2.4	7.23	5.050 ± 0.17*	×10 ³ /μl	5.5–19.5	14
	PCV	25.56	41.4	34.29 ± 0.55*	%	39–55	14
	Hb	10.1	19.5	13.44 ± 0.40*	gm/dl	9.8–15.4	4.2
	MCV	56.92	91.02	72.73 ± 1.43*	fl	40–60	20
	MCH	21.51	31.63	27.92 ± 0.44*	Pg	13–17	4
	MCHC	28.45	52.7	39.23 ± 0.98*	gm/dl	30–36	6
	PLT	252	410	318.3 ± 6.62*	×10 ³ /μl	300–800	500
Differential leukocytic count	Neutrophils	14.34	29.38	21.56 ± 0.66*	%	45–64	19
	Eosinophils	0.0	2.0	0.830 ± 0.10*	%	0–4	4
	Basophiles	0.0	2.0	1.00 ± 0.0 ^{NS}	%	0–1	1
	Lymphocytes	41.11	61.7	49.19 ± 0.73*	%	27–36	9
	Monocytes	2.0	9.0	5.50 ± 0.2 ^{NS}	%	0–5	5
Liver function markers	AST	34.2	128	46.85 ± 2.00*	U/l	39–79	40
	ALT	13.2	58.4	26.73 ± 1.75*	U/l	4–11	7
	ALP	21.8	85.6	42.90 ± 2.76*	U/l	10–77	67
	Albumin	2.2	2.9	2.55 ± 0.030*	gm/dl	2.8–3.8	1
	Total protein	6.2	8.8	6.96 ± 0.110*	gm/dl	6.3–8.9	2.6
Kidney function markers	T. bilirubin	0.1	1.21	0.23 ± 0.04 ^{NS}	gm/dl	0.1–0.4	0.3
	Urea	16.6	20.0	17.94 ± 0.12*	mg/dl	6.0–22.0	16
	Uric acid	1.18	2.6	1.64 ± 0.050*	mg/dl	1.9–7.5	5.6
	Creatinine	0.9	1.5	1.17 ± 0.020*	mg/dl	0.8–1.4	0.6
C.R. protein	CRP	24.0	96.0	35.25 ± 2.64*	μg/ml	1–6	5

All data are presented as mean ± standard error (SE) for 60 samples ($n = 60$) and statistically analyzed against the absolute range value (maximum reference range – minimum reference range) using the one-sample t-test. The differences were considered to be significant at $*p < 0.05$.

The hematological parameters of cattle exposed to the examined heavy metals including Al, As, Ni, Pb, Hg, and Cd showed a 50% and 65% decrease in the total WBC and RBC counts, respectively. Solitary cases showed Hb decrease, but no decrease had been recorded in Hb. However, MCV and MCH showed a marked increment in all the examined animals. The concentrations of MCHC were varied from decrement to increment. Furthermore, no changes in blood platelet counts had been detected. For leukocytic count, lymphocytosis and monocytosis were recorded. On the other hand, neutropenia was noticed in most of the exposed animals. No changes were found in the count of both basophils and eosinophils.

No changes were noticed in the concentration of urea, uric acid, and creatinine, and the obtained data were almost within the normal ranges. There were increased concentration levels in AST enzyme in every 10 cases of the exposed animals. However, increased ALT activity was detected in all animals. Increased total bilirubin was detected in one animal. Both albumin and total protein values were within the normal. Heavy metal toxicity sharply increased the serum C-reactive protein (CRP)

concentrations which indicate the inflammatory process inside the animal body. The concentrations of CRP depend on the severity of inflammation, where it appears very high in some animals approximately 96 mg/ml.

The linear regression and correlations of heavy metals detected in raw cows' milk such as Al, Ni, Pb, and Cd with their respective concentrations in feeding material and water ($n = 60$) are shown in Figure 1. All the concentrations of Al, Ni, and Cd detected in the feeding stuff and drinking water were significantly correlated to their respective concentrations in milk (Fig. 1 A, B, C), but Pb did not (Fig. 1D). However, the feeding stuff concentrations for Al, Ni, and Cd showed a strong correlation to their respective levels in milk rather than those detected in water ($R^2= 0.072$ vs. 0.039 , 0.13 vs. 0.10 , and 0.46 vs. 0.014 , respectively; $p < 0.05$).

The significant correlation matrix among the heavy metals detected in milk and those blood/biochemical parameters in cattle showed that Al and Ni are the most factors significantly correlated to the concentrations of RBCs, lymphocytes, creatinine, AST, and MCV. The correlations were evaluated by linear regression (R^2) ($n = 60$) (Fig. 2).

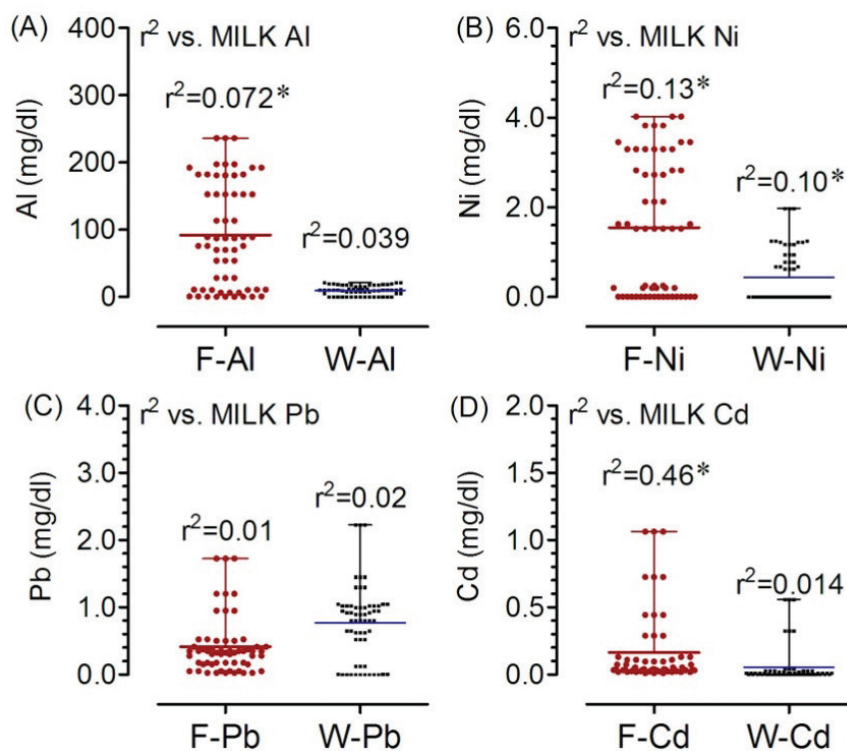


Figure 1. Linear regression of heavy metals such as Al, Ni, Pb, and Cd. The correlations of Al, Ni, Pb, and Cd concentrations in milk compared to their respective concentrations in feeding material (F-Al, F-Ni, F-Pb, and F-Cd) and water (W-Al, W-Ni, W-Pb, and W-Cd) for 60 sample categories ($n = 60$) are shown in Fig. 1 A, B, C, and D, respectively. The correlation was considered to be significant at $*p < 0.05$.

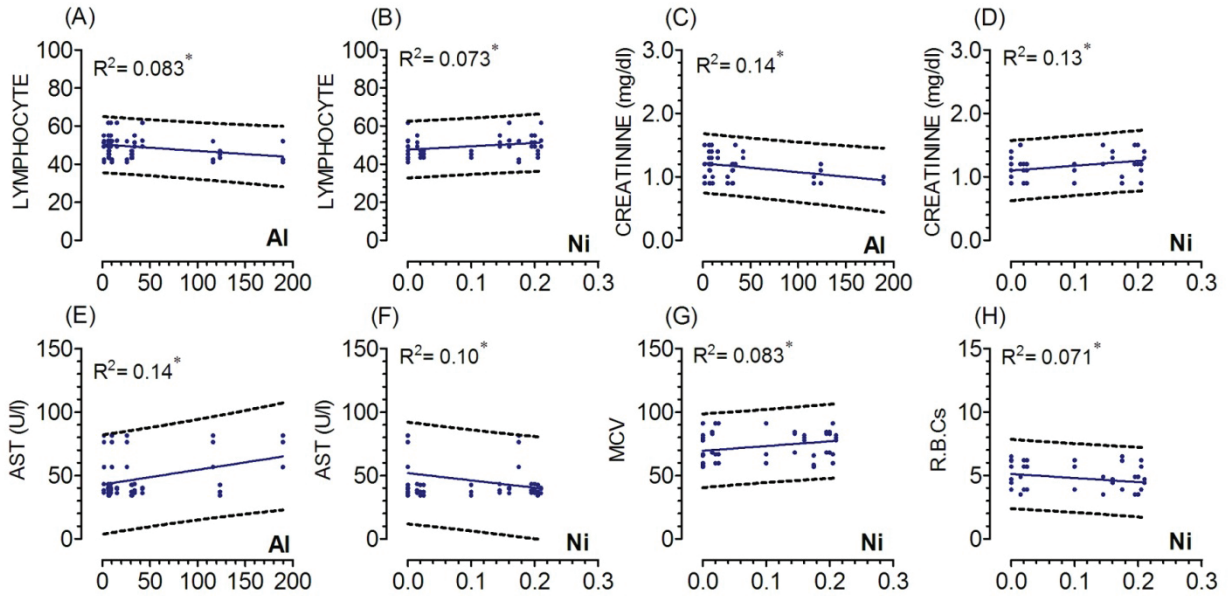


Figure 2. The significant correlation detected between heavy metals; Al and Ni in the raw cow's milk against blood/biochemical parameters were evaluated by linear regression (R^2) for 60 cases ($n = 60$). R^2 between Al versus lymphocyte, creatinine, and AST is shown in Fig. 2A, C, and E, respectively. R^2 between Ni versus lymphocyte, creatinine, AST, MCV, and RBC is shown in Fig. 2B, D, F, G, and H, respectively ($*p < 0.05$).

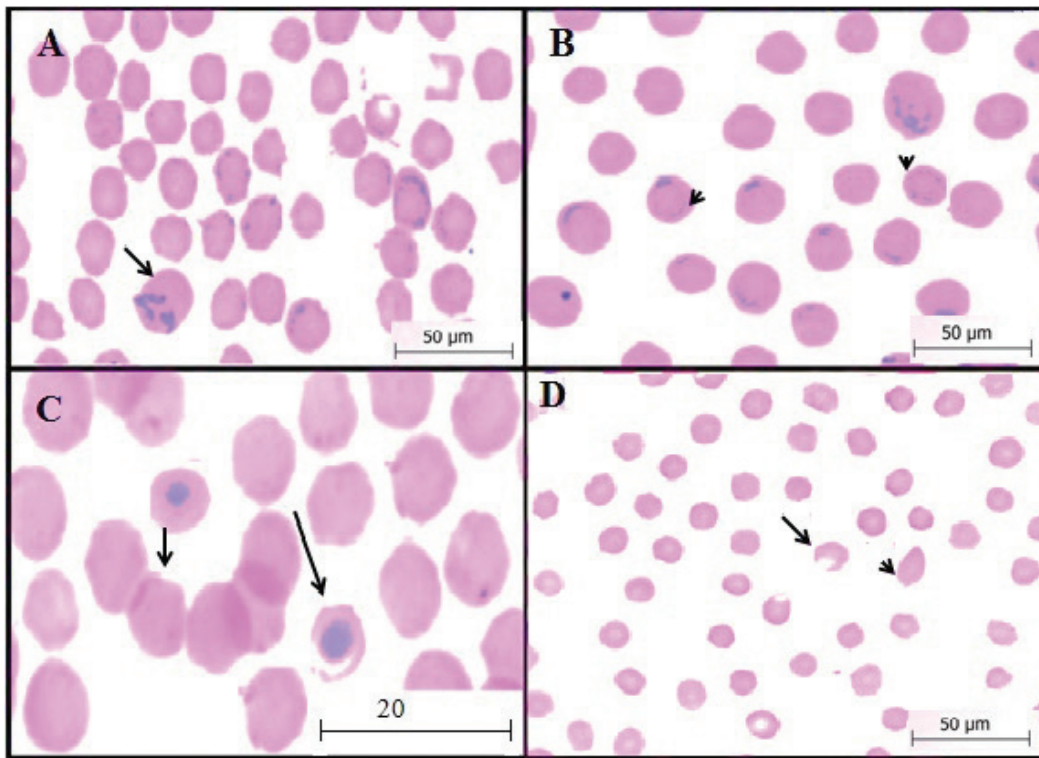


Figure 3. Blood film smear by Giemsa stain showing the effects of heavy metal exposure on red blood cell morphology; (A) reticulocytes (arrow), (B) basophilic stippling (arrow head), (C) nucleated red blood cells (long arrow) and tendency to the rouleaux formation (short arrow), (D) keratocytes (arrow) and elongated cells (arrow head).

Discussion

Heavy metals' pollution related to the industrial activities becomes a hazard to public and livestock health in several regions worldwide. The environmental traits of various localities in Egypt are the major important determinants of heavy metal hazards. Clarifying the heavy metal pollution magnitudes among the surveyed villages, the levels of exposure and the roles of various sources might provide insights to improve the environmental standards, additionally for assist to affirm the required innovative environmental management approaches to implement control measures for public health protection [20]. Assessing the exposure of the population to a distinct/mixture of heavy metals through various sources including drinking water, milk, and food concurrently was a valuable tool to measure the overall human environmental exposure [20]. During the current study, heavy metals load in water contributed more to the total adult human exposure levels of Al, Ni, Hg, Pb, and Cd compared to the other tested sources (Table 2B). The exposure contributions of water were 81.3%, 98.5%, 100%, 98.5%, and 98.5% for Al, As, Hg, Pb, and Cd, respectively, whereas the exposure contributions of milk were 18.7%, 1.3%, 0.0%, 1.5%, and 1.7% for Al, As, Hg, Pb, and Cd, respectively. Besides, the contributions decrease in the order of water > milk > feed for calves with 36.6%, 70.6%, 54.6%, 88.2%, and 63.3%; 31.9%, 5.2%, 44.3%, 7.2%, and 5.7%; and 28.5%, 24.2%, 1.1%, 4.6%, and 30.7%, respectively. In relation to age, there was a dependable pattern, in which the contributions of each source are as follows: water > feed for both growing heifers and lactating cows as shown in (Table 5B). Unambiguously, water sources in the surveyed regions have the potential to be the major contributor to the total heavy metal human environmental exposure. The literature based on other studies conducted in many countries globally revealed that water could play the utmost role for the overall heavy metal exposure among human [20].

The examined polluted drinking water samples contain four heavy metal concentrations exceeding the permissible limit approved by the WHO arranged in order as follows: Cd (77%), Pb (80%), Hg (87.5%), and Al (93.8%). The current results agreed with former findings reported by researchers in Egypt, and they found heavy metals exceeding the permissible limit in water when matched to those predetermined by the WHO and US-EPA [21]. Other reports stated that although the examined drinking water showed the levels of some heavy metals just equivalent to their respective MPL, chronic exposure of animals and human to such water leads to a wide diversity of adverse clinical conditions affecting the animal and human health [22,23]. All examined water samples were positive for Pb pollution with levels greater than the MPL of the WHO which agreed well with some Egyptian researchers who

determined the same Pb concentrations in drinking water sources in different areas in Egypt [21]. It is known that drinking water plays a clear role as sources for livestock and human toxicity with various mixtures of heavy metals which could have serious implications on veterinary and public health in Qena Governorate, Egypt. This is in agreement with another study conducted on great Cairo cities, Egypt, which demonstrated that a robust association between heavy metals polluting the drinking water and diseases such as kidney failure, hepatic cirrhosis, and anemia was reported [21].

It is clearly noticed that heavy metal pollution levels among cattle feeding material primarily reflect their harmful effects on the animal products that could be bioaccumulated and released from the animal body through animal products, such as milk, and therefore, enter into the human food chain [5,24]. The study highlighted that all the analyzed samples of cattle feeding stuff were positive for all heavy metals examined, including all mentioned for drinking water plus Ni and As with 70% and 85% exceeding their MPL. It is found that Al is the most polluting heavy metal in both drinking water and animal feedstuff (93% and 90% > MPL, respectively). This suggests that the feeding stuff used as animal ration in Qena, Egypt, might have a unique presence and distribution of the wide diversity of heavy metal. In comparison, about 28% of all examined feeding material samples were polluted with at least one heavy metal above EU limits, and those ruminant feeds showed the elevated levels of pollution [24]. The study is consistent with an Indian report which studied the grasses as a fodder for cattle and found the higher levels of pollution with Cd and Pb compared to their MPL [25]. Those variations between these results and former findings from other countries might be attributed to the diverse levels of heavy metals among feeding materials which were formulated from a variety of ingredients and exposed to different degrees of pollution, either during processing, transportation, or storage. Furthermore, cattle feed may be prepared from the grasses and forages which irrigated with industrial and municipal wastewater-polluted heavy metal [25].

Raw milk plays a crucial role as a source for human and animal toxicity with various mixtures of heavy metals having serious hazards on the public health due to their deposition in the adipose tissue, which leads to their bioaccumulation and biomagnification. Therefore, their presence in milk is cumulative regardless of the small amounts or equivalent to the MPL and so their hazards appear on the long run.

The examined samples of raw milk showed variable percentages of heavy metal pollution for 60 samples, such as 5% (Hg; $n = 3$), 65% (Ni; $n = 39$), 95% (Cd; $n = 19$), and 100% (Al; $n = 60$, and Pb; $n = 60$). Furthermore, those heavy metals exceeded their MPL in variable percentages,

such as 100% (Hg), 15.4% (Ni), 15.8% (Cd), and 100% (Al and Pb). Those values are in agreement with the results reported by other Egyptian researchers who stated that heavy metals were detected in raw milk samples in various levels more than the permissible limit set by EOSQC, Codex Alimentarius, IDF [26]. Moreover, it was stated that the concentrations of heavy metals were more than the maximum limits approved by the national and international organizations [5]. The greater pollution of the environment, especially from effluents, emissions from industries and vehicles, and use of pesticide Pb to increase in the loads of heavy metals in water, air, soil, and plants, in which animals' intake leads to the pollution of milk [27]. The present study stated the significance of the polluted raw milk as a potential source for heavy metal hazards for both human and animal in Qena Governorate, Egypt.

Milk usually contains very low Al concentration except when cows have ingested feeding materials or drinking polluted Al-containing canal water or feeds [28]. However, Al concentrations in examined raw milk are higher than those of drinking water, indicating that the pollution of milk is not dependent on drinking water as a unique source. The study detected Al in 100% of feeding material samples (92.05 ± 0.10 mg/kg), giving evidence that the main contribution might have come from polluted feeding material. A large amount of Al pollution could result from the abundant and traditional use of Al-made containers of improper design and bad quality materials for milk processing and storage [29]. The storage of milk for long time in Al-made containers give an opportunity for Al to be released into the milk [30] or even milk adulteration with Al-containing water [31].

The study revealed several changes of hematological and biochemical in response to the heavy metals' toxicity, including the following: (a) anisocytosis (macrocytes) associated with increased levels of MCV and MCH. The larger sized blood cells due to Vitamin B12 malabsorption as a result of disturbing of heavy metals absorption in the gut [32], (b) poikilocytosis with prominent keratocytes resulted from the oxidative stress induced by metal toxicity effect on the fragile cell membrane [33], (c) reticulocytes which indicate bone marrow activity in response to blood-losing anemia and the inflammatory hemorrhage due to heavy metal toxicity, (d) the tendency to rouleaux formation which refers to inflammatory process causing increased plasma protein (fibrinogen) level and so decreasing the repellent power between RBCs and that the surface negative charge [34], and finally (e), the basophilic stippling is a pathognomic finding for heavy and trace metal toxicity, especially the Pb which prevents the ribosome-degrading enzyme 5-nucleotidase [35].

Heavy metal toxicity induced macrocytic anemia evidenced by decreased RBC count associated with increased

levels of MCV and MCH [36]. Heavy metal intoxication also induced immune suppression evidenced by leukopenia or decreased WBC count, in about 50% of the examined animals. These findings were also detected in mice treated with Pb [37]. Anemia has several causes including the destruction of RBCs [38] by decreasing the synthesis and liberate of RBCs into the bloodstream [39]. Thus, heavy metal toxicity is a direct suppressing factor of the hemato-poietic activity in the living body [40]. In association, heavy metal toxicity exhibited lymphocytosis, monocytosis, and neutropenia due to acute and chronic toxic inflammation in various organs in response to trace metal toxicity. Moreover, lymphocytes, together with monocytes, are associated with the different forms in the inflammatory processes [41]. Besides, T-lymphocytes play a protective secretory role in the inflammatory response associated with tissue affections, such as liver cirrhosis, rheumatoid arthritis, and atherogenesis [42]. Exposure of the animals to heavy metals induced the increases in AST enzyme activity in two out of 60 animals while that ALT increased in all animals. Liver enzymes' activities were due to the role of the livers in the detoxification of some metals such as Al, Ni, Hg, and Pb [43] toxicity. The main heavy metal implication is oxidative stress. Metals augment the lipid peroxidation defenses in tissues by the alteration of the antioxidants, such as catalase, superoxide dismutase, and glutathione peroxidase [44].

C-reactive protein (CRP) is known to rise in response to a wide range of inflammatory conditions either acute or chronic as well as tissue injury and necrosis in response to the released cytokines, i.e., interleukin-6 that enhances the synthesis of CRP and fibrinogens by the liver [45]. Heavy metal toxicity stimulated the CRP release with variable degrees in all animals in response to the inflammatory effects induced by heavy metal intoxication. Both lymphocytosis and monocytosis in exposed animals are required for CRP function since interleukin-6 released by T-lymphocytes and macrophages is important for CRP synthesis by activating the complement system via the C1Q complex [46]. The long-term consumption of polluted drinking water, milk, and feeding material with heterogenous heavy metal mixture could result in wide adverse health effects and diverse range of pathological affections including cancer, mental/nervous system damage, impaired genital/reproductive function, hematotoxicity, allergic disease, hepatic injury, renal damage, gastrointestinal disturbances, persistent restlessness, skin disorders including long-lasting rashes, and hair loss and could interfere with the immunocompetence of consumers due to the synergistic effect between heavy metals [47]. The heavy metals causing anemia as found in our study and consistent with others are Pb, Cd, As, and Hg [48,49].

THQ value far above 1 highlighted that both children and adult drinking from water sources in the studies villages expected to suffer from noncarcinogenic health hazards due to heavy metal pollution, while HQ value above 1 for Al and Cd in milk indicated that ingestion of cow's milk for children considered as a risk for their health. The HI data indicative of an elevated tendency to develop non-cancer-related diseases among both adults and children due to the exposure of heavy metal mixture detected elements in water sources and cow's milk. The TCR values reveal a high threat for people living in those villages, which predispose them to undergo some types of malignancy, since many of the recorded values are more than the maximum values of 10^{-6} – 10^{-4} as given by the US-EPA (2002) [14].

The study faced some limitations including sample size and diversity. Furthermore, the study initiated in rural village area while excluding urban regions. A future study should be conducted on a large-scale sample size covering a wider geographical area. A comparative analytical study of the levels of heavy metal pollution should be performed between rural and urban areas. A diversity of samples should be broadened to have more environmental compartment such as air, soil, and also more foodstuff of both human and animal origins.

Conclusion

Human and dairy cattle are suffering from chronic exposure to heterogeneous heavy metal mixture originated from drinking water, milk, and feeding materials and exceeding the MTDL, ADI, and MPL stipulated by international organizations. It is appeared to have a unique presence and distribution of the wide diversity of heavy metal in Qena, Egypt. Majority of cattle suffered from anemia, immunocompromised state, and impaired liver function. The present study states that heavy metal pollution could be the major cause of the present health hazards detected in animals and human beings. Innovative strategies are crucial to implement one health concept to determine the health requirements of those targeted villages' communities through notifying the findings of the current study to relevant public health decision-makers and veterinary and environmental authorities to take the necessary counteract measures.

Acknowledgment

The authors would like to express their gratitude to South Valley University, Egypt, for supporting the study.

List of Abbreviations

ADI, Acceptable Daily Intake; AI, Acceptable intake; Al, Aluminum; As, Arsenic; ALP, Alkaline phosphatase; ALT,

Alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood Urea Nitrogen; Cd, Cadmium; CRP, C-Reactive protein; EDI, Estimated Daily Intake; EDL, Estimated dietary level; EI, Estimated intake; EOSQC, Egyptian Organization for Standardization and Quality Control; EU, European Union; EWI, Estimated weekly Intake; Hb, Hemoglobin concentration; Hg, Mercury; HI, Hazard Index; HQ, Hazard Quotient; ICP-OES, Inductively coupled plasma optical emission spectrometry; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; MCV, Mean corpuscular volume; MTDL, Maximum Tolerable Dietary Level; Ni, Nickel; OIE, The Office International des Epizooties; Pb, Lead; PCV, Packed cell volume; PTWI, Provisional Tolerable Weekly Intake; RBC, Red blood cell count; TCR, Target cancer risk; THQs, Target Hazard Quotients; WBC, White blood cell count.

Conflict of interest

The authors do not have a conflict of interest.

Authors' contribution

HMD and ASA conceived the ideas and study experimental design, processed the experimental data, and wrote the manuscript. AAM, AEA, AAS, AAE, AMK, MAA, and IFR were involved in data analysis and interpretation and contributed to the final manuscript.

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Supplementary Material

Table S1. Parameters and variables used in the calculation of THQ and TCR.

Parameter	Unit	Child	Adult	References
E_{FR}	Days	365	365	Kamunda et al. [1] Antoine et al. [2]
ED	Years	70	70	Kamunda et al. [1] Antoine et al. [2]
F_{IR}				Nutrition Institute, Cairo [3] Nutrition Institute, Cairo [4] Shaw [5]
Milk	ml/day	300	200	
Water		1100	3000	Dietary Reference Intakes [6]
BW	kg	25	65	Nutrition Institute, Cairo [3] Nutrition Institute, Cairo [4]
ATn	Days	365×8	365×30	Kamunda et al. [1] Antoine et al. [2]
ATc	Days	-	365×70	Kamunda et al. [1] Antoine et al. [2]
RfD				
Al			1.0	Antoine et al. [2]
As			0.0003	Castro-González et al. [7]
Ni			0.02	Castro-González et al. [7]
Hg	(mg/kg)		0.0003	Kamunda et al. [1]
Pb			0.036	Harmanescu et al. [8] US-EPA [9]
Cd			Milk: 0.001 Water: 0.0005	Harmanescu et al. [8] US-EPA [9] Kamunda et al. [1]
CPSo				
Ni	mg/kg day ⁻¹		1.7	US-EPA [10]
Pb			0.0085	US-EPA [10]
Cd			0.38	US-EPA [10]

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