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DETERMINATION OF HEAVY METALS CONCENTRATION IN RAW MILK OF AWASSI SHEEP GRAZED IN THE POLLUTED AREA BY OIL REFINERY EFFLUENTS IN KWASHE INDUSTRIAL AREA

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ABSTRACT

Nowadays, heavy metals are regarded as the most significant contaminants due to industrial activities and have an impact on their presence in milk. The presence of heavy metals in milk could have a serious negative impact on public health. The current study aims to determine the amount of various heavy metals (Pb, Cu, Co, Cd, Cr, Ni, Fe, and Zn) in raw Awassi ewe's milk collected from Kwashe industrial area, Duhok province, Kurdistan Region, Iraq. The sheep were grazed in Sulaivany plain contaminated by drains of industrial effluents of several crude oil refineries and other 200 different factories. Atomic absorption spectrophotometer was utilized to analyze the milk specimens after wet digestion. Results displayed that the heavy metals concentration was in the range of 3.64-4.27 mg/L for Pb, 0.59-1.13 mg/L for Cu, 0.01-0.09 mg/L for Co, 0.12-1.46 mg/L for Cd, 0.24-0.29mg/L for Cr, 0.89-0.99 mg/L for Ni, 0.89-0.94 mg/L for Fe and 3.99-6.13 mg/L for Zn. Statistical analyses showed that excusing Cr, and all other studied heavy metals concentrations were higher than the human-health safety recommended. Furthermore, among the heavy metals in the current study, the Zn was the highest mean value recorded (4.99 mg/L) and it exceeded the limited value (3.8 mg/L) by WHO/FAO, (1999). The permissible level of pb in milk is 0.05 mg/L, and the mean concentration of pb in the milk sample (3.99 mg/L) was significantly higher than the permissible value. Moreover, the mean value of Cd (0.75 mg/L), Cu (0.79 mg/L), Co (0.038 mg/L), Ni (0.948 mg/L), and Fe (0.91 mg/L) were above the limited value (0.01 mg/L) for Cd, 0.03 mg/l for Cu, (0.001-0.008) for Co, (0.1mg/L) for Ni and 0.1 for Fe, respectively. While the Cr concentration (0.27 mg/L) was lower than the recommended level (0.3 mg/L). Therefore, it was observed that the amount of heavy metals in the sheep milk utilized in this investigation possesses a health risk. Hence, it is always needful to persuade the pollutants in milk in the current area.
KEYWORDS: Heavy metals, Milk, polluted area, oil refinery, sheep, Kwashe industrial area

INTRODUCTION

Well-known as minor elements, heavy metals are common environmental substances that can have natural (such as volcanic) and anthropogenic (such as industry) sources. Therefore, increased industrialization is directly connected with rising levels of environmental contamination (Stanovič et al., 2016).

Heavy metals, as toxic metals in the environment, have recently been discovered all over the world (Rafiq et al., 2022). Generally, the name "heavy metals" depicts a class of micro-metals, metalloids, and their derivatives that have a high density and are toxic at even small concentrations (Sonone et al., 2020). Due to their toxicity, persistence, and bioaccumulation issues, heavy metals have recently become one of the more harmful contaminants in the human environment (Ariyaee et al., 2015). The majority of heavy metals can be hazardous to all life forms, including bacteria, plants, animals, and humans in large quantities, although some of them are necessary to trace elements (Abdullah et al., 2019; Alnabi et al., 2022).

According to their effectiveness and intoxication, heavy elements are divided into two classes. Some of them are toxic even with low concentrations which are directly hazardous to living things like As, Cd, Cr, Hg, and Pb. Meanwhile, Cu, Fe, Mn, Ni, and Zn are important elements that living organisms must have in modest quantities and their insufficient consumption results in deficient symptoms. However, in greater quantities, they might cause poisoning (Khan et al., 2019). Substantial quantities of heavy metals could be transported from polluted land, water bodies, and ambient to plants and meadows, resulting in the cumulative levels of these metals in pasturing livestock and their subsequent conveyance to the food chain through the milk and the meat. Consequently, the buildup of serious metals in livestock not just results in harmful impacts on the productivity of cattle, but causes sanitary hazards to people too, particularly those who consume milk and meat that were spoiled with the toxic metals (Gupta et al., 2021).

Recently, the contamination of milk by heavy metals is considered a public health concern that has a detrimental impact on the community's health, particularly that of children (Chirinos-Peinado & Castro-Bedriñana, 2020; Rafiq et al., 2022). It is generally recognized that increasing urbanization and industrialization have contributed to higher concentrations of heavy metals, which eventually find their way into milk and dairy products with different pathways for instance, through the contaminated feed of animals and industrial effluents surrounding regions where animals are being fed (Iftikhar et al., 2014; Chandrakar et al., 2018).

Previously, several investigations were undertaken by several researchers such as Ogabiela, et al., (2011) in Nigeria, Lutfullah et al., (2014) in Pakistan, Rezaei et al., (2014) in Iran, and Abdel Khalek, et al., (2015) in Egypt who found a range of toxic metals in milk, most of which were much above allowable levels.

Milk is a key ingredient in the manufacture of numerous food items. Moreover, it is essential nutrition for the child, it must be an acceptable quality and heavy metal levels should be carefully controlled. Therefore, this study aimed to determine the

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concentration of several heavy metals in Awassi ewe milk collected from a farm in the Kwashe industrial area (KIA) to estimate the impact of these heavy metals on public health.

MATERIALS AND METHODS

Study Area

The location of the study area was in the KIA at Summel district, Kurdistan Region, Iraq (36°59'04.2"N 42°47'50.8" E), 25 kilometers Northwest of Duhok province. This industrial area contains approximately 200 factories, with fifty crude oil refineries. About a million liters of untreated effluent are released into the area daily (Meshabaz & Umer, 2022). Therefore, this area is continuously contaminated with various pollutants, especially heavy metals.

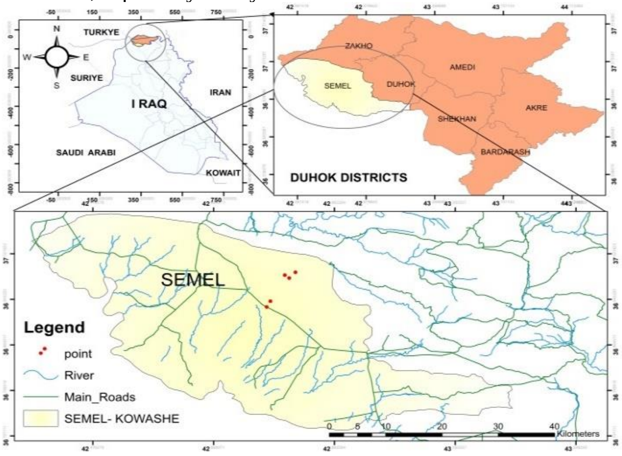

Figure (1): The location of the study area and site sampling

Collection of samples

Samples of raw milk were collected from 35 Awassi ewes during the lactation period in 5 different farms located in KIA that depend on effluents and forages grown in contaminated soil with effluent as a source of drinking water and feeding. All milk samples were taken according to Italian Official Analytical Methods, 1986 (no metallic containers, etc.) to reduce potential external contaminations. An aliquot was conserved at -18°C to be subsequently analyzed. The milk samples were defrosted at room temperature right before the examination and then they were filtered, homogenized, and processed analytically.

Preparation and digestion of samples

The sample preparation process consisted of four sub-steps including the acid digestion of the samples, filtration of the samples, dilution of the digested samples, and finally analysis of heavy metal.

Acid digestion of milk sample based on the procedure outlined by Finerty et al. (1990), 1 mL of sample was blended with 10 mL 3:2 HNO_3 (65%v/v): $HClO_4$ (70%v/v). Then, it was digested at 90°C on an electric hot plate and this mixture's temperature was gradually raised to 120ºC while being stirred every 30 minutes to allow improved digestion. The final volume of each sample was raised to 50 mL after filtering the digests, and it was then kept in plastic bottles until analysis.

Analysis of samples

The concentrations of all heavy metals presented within ingested samples were determined by atomic absorption spectroscopy (Perkin–Elmer, PinAAcle 900 AAS Consumables and Supplies). The blank for the sample analysis was created, and the calibration standards were created by diluting multi-element standard solutions at a concentration of 100 mg/l. The responses were measured after each atomization of the blank and the standards. The sample solutions were atomized and tested after a calibration graph was generated for each of the solutions. Based on the absorbance measured for the unknown sample, the calibration was used to determine the various metal concentrations from the sample solution.

Statistical analyses

The collected data were submitted to SPSS software (SPSS, 2019), to analyze them statistically. According to the Shapiro-Wlik normality test, the data of heavy metals were normally distributed; therefore, they were analyzed using a parametric ANOVA test. The Chi-square test was applied for testing the observed content of heavy metals compared to its expected standard ranges/values, in the samples of the studied objectives.

RESULTS AND DISCUSSION

The levels of trace metals in the samples analyzed are demonstrated in Table (1). The heavy metals concentration was compared to the acceptable limits in the milk of farm animals according to WHO/FAO, (1999). The levels of heavy metals in the results were in the following magnitude order: Zn=Pb > Ni>Fe > Cu > Cd> Cr>Co (max to min). The maximum amount of heavy metals contained in Awassi sheep milk is due to their direct dependence on heavily contaminated industrial effluents as the main source of drinking water that flows in Sulaivany grazed plain without any kind of handling or treatment, as well as the grown forage in contaminated soil by these effluents. Large quantities of heavy metals were found in milk along with other various dairy products in contaminated soil (Kazi et al., 2009; Soylak et al., 2005).

As displayed in Table (1), the Pb concentrated in milk ranged from 3.64 to 4.27 mg/L with a mean value was 3.99 mg/L. The acceptable amount of Pb in milk is 0.02 mg/L of fresh milk. It could be noticed that Pb concentrations in the present work were 10-fold over the allowable limit as illustrated in Fig. (2). Therefore, the consumption of this milk by a human should be banned as it is associated with high carcinogenic risks, particularly in children. Additionally, Pb poisoning is a toxic condition created from Pb probation. In both children and adults, Pb intoxication preliminary influences the digestive system and central nervous system (Engwa et al., 2019). Pb residues in water and animal feed are the most frequently reported source of Pb contamination in milk. The Pb concentration in present milk was greater than was found by Póti et al. (2021). Furthermore, Pb concentration in milk from the industrial area was shown to be statistically higher than in milk from a location supposed to be non-industrial pollutants by Dobrzaski et al. (2009).

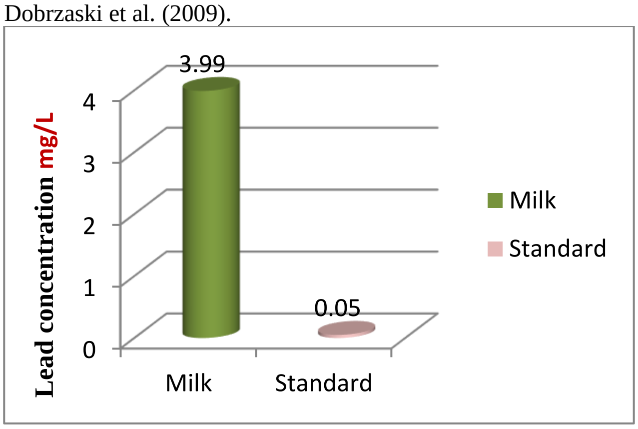

Fig (2): the concentration of Pb in milk samples in comparison to the standard value

The minimum level of Cd was 0.12 mg/L and the maximum level was 1.46 mg/L while the mean average Cd concentration in milk was (0.75 mg/L). It is obvious from obtained data that all samples of raw sheep milk had Cd concentration above the allowable limit (0.01 mg/L) as recommended by WHO/FAO, (1999) (Fig. 3). Consumers are at risk in this circumstance, especially children as Cd is an active carcinogenic agent as Pb. Furthermore, studies on humans and animals showed that Cd can cause osteoporosis (skeletal deterioration), which can lead to bone mineralization (Engwa et al., 2019). The existing findings were greater than the findings of other studies undertaken in highly contaminated regions of other nations such as the peak amounts of Cd in milk confirmed in Ecuador, Egypt, Italy, Mexico, Pakistan, and Romania were 0.46, 0.11, 0.02, 0.29, 0.06 and 0.01 mg/L, successively (Chirinos-Peinado, and Castro-Bedriñana, 2020). Moreover, the amount of Cd (52.9µg.kg-1) was found in the sheep milk from the Poráč area in a study conducted by Stanovič et al. (2016). The primary source of Cd contamination in the study environment is a particulate matter from the metal smelting that enters wet and pasturing areas mostly during the wet season, travels to meadows, then enters the animal blood and milk where it binds with lipids and proteins including casein and whey proteins (Chirinos-Peinado & Castro-Bedriñana, 2020).

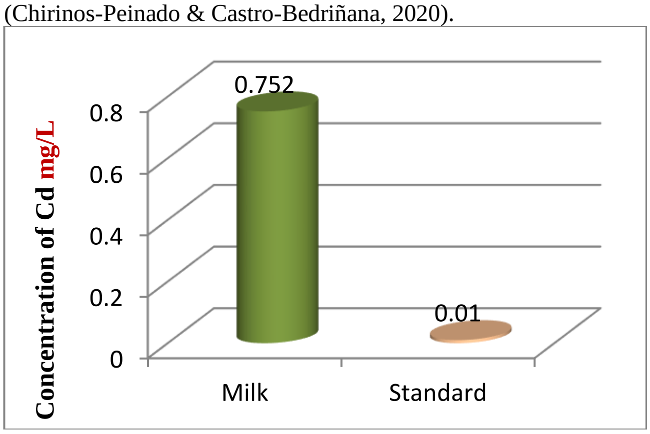

Fig (3): Concentration of Cd in milk sample samples in comparison to the standard value

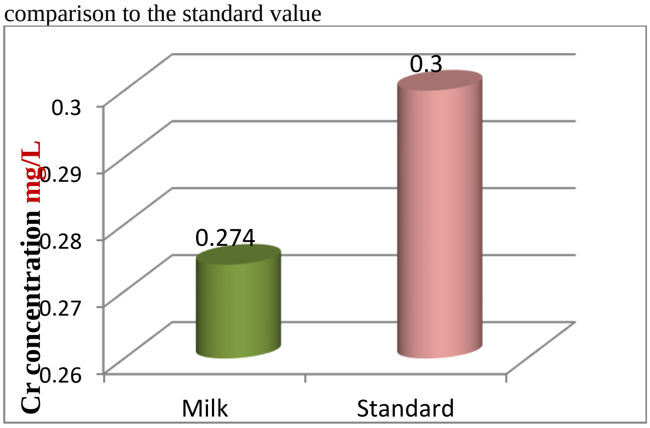

Fig (4): the concentration of Cr in samples in comparison to the standard value

The minimum and maximums Cr levels in the present study were (0.24 and 0.29 mg/L) respectively, while the mean concentration was (0.27 mg/L). A similar value of Cr 0.29 in sheep milk was proved by Póti et al. (2021). The Cr quantity in the current work was within the permissible level (0.3) (Fig. 4). It is conclusive that concerning the Cr concentration, consuming this sheep's milk is entirely safe without creating a health risk.

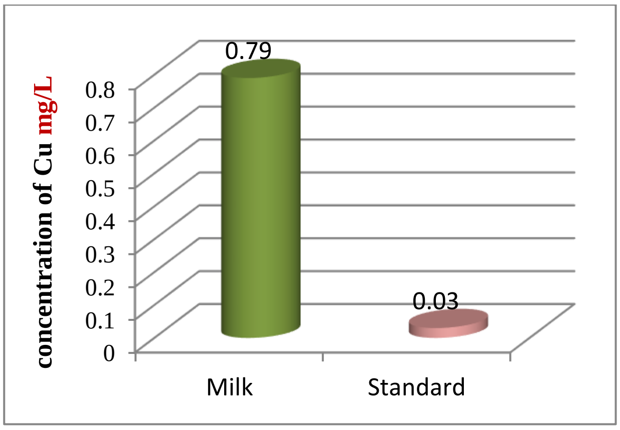

Fig (5): the concentration of Cu in samples in comparison to the standard value

The appropriate amount of Cu and Zn in meals is desired because they are generally considered necessary micronutrients and act as enzyme cofactors in metabolism. Comparatively low quantities of Cu, as compared to Zn, were found in the examined milk samples. The mean average value of the Cu content was 0.79 mg/L as this value was above the limited value of 0.03 mg/l WHO/FAO, (1999) (Fig. 5). Similarly, higher concentrations of various heavy metals were found in milk and cheese samples gathered from the metal-affected site, it was discovered that the Cu concentration in the milk samples on the roadside was importantly bigger than in areas secured from pollution (Rasheed et al., 2022). In addition, El-Badry and Raslan (2016) recorded 0.43 mg/L of Cu in sheep milk. Generally, agriculture and various mining and smelting operations are the main causes of contamination of the area with Cu. Among the heavy metals in the current study, the Zn was the highest value recorded (4.99 mg/L) and it exceeded the limited value (Fig. 6). Current findings were consistent with those confirmed in Poland (5.53 mg/L) (Stanovič et al. 2016) and in Turkey (5.02 mg/L) (Paksoy et al., 2018) and greater than values reported in Pakistan (Khan et al., 2006) and Croatia (Antunović et al., 2016).

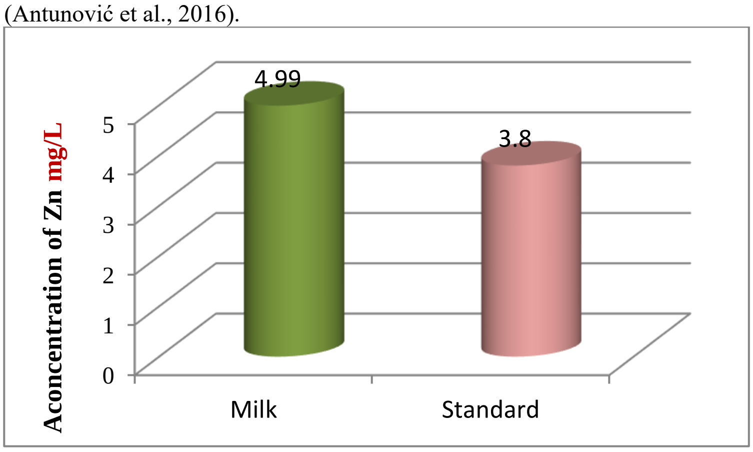

Fig (6): the mean concentration of Zn in milk samples in comparison to the standard value

The concentration of Co in the present work varied from 0.01 to 0.09 with a mean range was (0.038 mg/L). Current values exceeded the permissible range (0.001-0.008) as recommended by FAO/WHO (1999) (Fig. 7). This finding agreed with the finding of Safonov (2020). The higher heavy metal content in milk samples is ascribed to the utilization of metal-polluted grazing land, water resource, and livestock pastured on metal-contained lands.

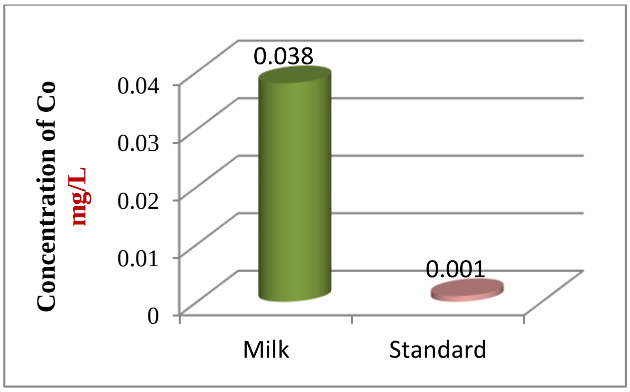

Fig (7): the concentration of Co in milk samples in comparison to the standard value

The level of Ni in the current research was ranging from 0.89 to 0.99 with a mean level of 0.948 mg/L. These data were higher than the recommended safe levels (0.1mg/L) as shown in Fig. 8. This result is higher than the findings of Saber and El Hofy (2018), who found 0.038 mg/l of Ni in sheep milk. Ahmad et al. (2016) indicated that the mean average value of Fe and Ni in examined milk were 0.592±0.321 and 0.34±0.001 mg/L, successively. Animal species, geographic location, maternal age, health state, lactation stage, and external factors including environment, diet, and season can affect the element concentrations in milk samples (Paksoy et al., 2018).

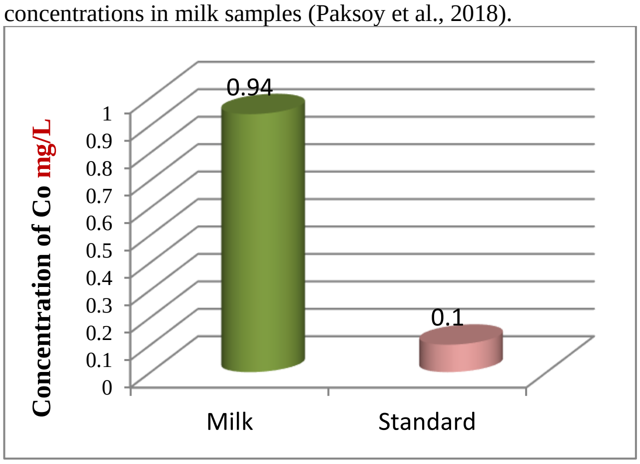

Fig (8): the concentration of Ni in milk samples in comparison to the standard value

The Fe value in the current study was (0.89-0.94) with a mean level was (0.91 mg/L) were exceeded the recommended level (Fig. 9). The current result was higher than the concentration observed in previous findings of 0.85 mg/L (Miedico et al., 2016) and 0.46 mg/L (Zhou et al., 2016). However, this average value was lower than that displayed by Paksoy et al. (2018), who observed that the Fe value presented within the milk samples was ranging from 2.83 to 4.72 with a mean value of 3.49 mg/L. The samples were collected during outdoor breeding and grazing. Thus, seasonality, atmospheric factors, and feed ratio fluctuation can all have a significant impact on the amount of Fe present in the sheep milk samples (Rahimi, 2013). The toxicity of iron encompasses four phases, first phase symptoms, starting six hours post-iron overdose, include nausea, vomiting, diarrhea, and bleeding in the gastrointestinal tract. The second phase develops 6–24 hours after an overdose, and this period is regarded as a chronic phase of obvious physiological improvement. The third phase commences from 12 to 96 hours following the outbreak of clinical signs and is marked by hypotension, shocks, drowsiness, hepatic necrosis, tachycardia, and lactic alkalosis, and may occasionally result in death. The last stage happens between 2-6 weeks after an overdose of iron. This phase is characterized by the emergence of gastrointestinal ulcerations and strictures (Engwa et al., 2019). It is shown from Table (1), some measured heavy metals such as (Pb, Co, Ni, and Fe) are differed significantly (p<0.01) between the milk of the studied ewe samples and the global standard range or values. This means that such milk from these toxic metals possess health risks to consumers and should be treated from the mentioned heavy metal, because it may cause fatal diseases.

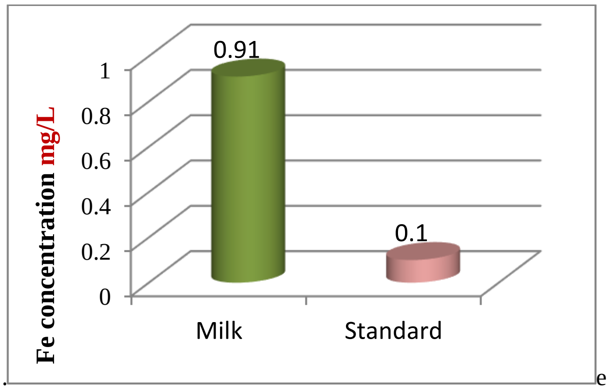

Fig (9): the concentration of Fe in milk samples in comparison to the standard value

Table (1): Descriptive statistics and Chi-square values of the studied heavy metals Concentration (mg/L) in the milk of Awassi ewe that grazed in the oil refinery polluted area

Heavy metals	Mean	Std. Deviation	Minimum	Maximum	Standard Range recommended by (WHO/FAO, 1999)	Chi-square value	Sig. (p)
Lead	3.998	0.23	3.64	4.27	0.05	6.2	**
Cupper	0.796	0.20	0.59	1.13	0.03	1.8	NS
Cobalt	0.038	0.03	0.01	0.09	0.001-0.008	5	*
Cadmium	0.752	0.53	0.12	1.46	0.001	0.2	NS
Chromium	0.274	0.02	0.24	0.29	0.3	1.4	NS
Zinc	4.996	0.89	3.99	6.13	3.8-5	1.5	NS
Nickel	0.948	0.04	0.89	0.99	0.1	5	*
Iron	0.908	0.02	0.89	0.94	0.1-0.7	5	*

** is highly significant (p<0.01); * is significant (p<0.05); NS is non-significant.

CONCLUSION

This study concluded that the concentration of all heavy metals in the milk of Awassi sheep grazed in the contaminated area with the oil refinery was higher than the limits allowed by international standards, except Cr which was below the standard level. It is possible to claim that consuming sheep milk in the present study's area is a health danger due to the heavy metal concentration. It could bring about long-term health hazards associated with consuming this sheep's milk. Consequently, it is essential to continuously monitor and control the feed and water of sheep to reduce the pollutants in milk in KIA.

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