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Polarographic Evaluation of Lead and Cadmium in Livers of Sheep in Zanjan and Sanandaj Cities, Iran

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ABSTRACT

Background: Lead and cadmium can enter the human body through consuming the liver of animals such as sheep, which may accumulate and cause adverse effects. The levels of lead and cadmium in the liver samples of slaughtered sheep in Zanjan and Sanandaj were investigated in the summer of 2014.
Methods: 96 Samples were collected from both cities. Lead and cadmium levels were measured by anodic stripping voltammetry (ASV) after digestion with nitric acid and hydrogen peroxide in 3 steps by a 400-watt microwave digester. Lead and cadmium concentrations were determined in µg/L using standard solutions of lead and cadmium as standard addition in their specific potential, -0.42 and -0.58 V for lead and cadmium, respectively.
Results: Mean values of cadmium in the livers of sheep in Zanjan and Sanandaj were 0.42 ± 0.13 and 0.35 ± 0.13 mg/kg, respectively, which had no significant difference ($P < 0.05$). On the other hand, mean concentrations of lead in the livers of sheep in Zanjan and Sanandaj sheep were equal to 3.69 ± 1.43 and 2.36 ± 0.18 mg/kg, respectively, which had a significant difference ($P < 0.05$).
Conclusion: The concentrations of lead and cadmium in the livers of sheep were higher than WHO standards. However, only the lead levels were higher than the maximum residue levels (MRLs) standard lists. The obtained results can be due to the activities of lead industries and factories in both cities, which may increase pollution levels in the water, air, and animal feed.

1. Introduction

Although some elements in the body may be essential to support human life in terms of nutrition and food metabolism, human exposure to their high or severe levels can have devastating effects on the body and pose grave risks to human health [1].

In this regard, the accumulation of cadmium (Cd) and lead (Pb) in various animal tissues, e.g., meat, liver, kidney, and other organs, may also be toxic for humans at different levels

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and endanger human health [2, 3]. In general, different levels of Cd and Pb accumulated in most foods are insignificant; however, higher levels are found in kidney and liver tissues and lower concentrations in the muscles of animals exposed to these elements. Therefore, consumers with relevant diets may be exposed to unacceptable levels of Cd and Pb [4]. Maximum residual levels (MRLs) of Pb and Cd in meat and farm-raised products have been set by the European Union (EU) Joint Commission, and routine food surveillance programs are carried out in the EU Member States to ensure that their levels will not exceed the determined MRLs. At present, MRLs set by the EU Joint Commission for Pb in the meat and offal (liver and kidney) are equal to 0.1 and 0.5 μg/g of wet weight, and for Cd in the meat, liver, and kidney, equal to 0.05, 0.5, and 1.0 μg/g of wet weight, respectively. Hence, foodstuffs not complying with the determined MRLs in the regulation must not be consumed or marketed as food [5].

Side effects and toxicity of Pb depend on the concentration, short-term and long-term exposure of an organism to Pb may vary from enzyme inhibition to relevant diseases and even death. Some researchers reported that Pb exposure had adverse health effects in both children and adults, which often involves central nervous system disorders in children, while other complications such as neuropathy, chronic nephropathy, and hypertension, are problematic in adults [6]. Pb enters the body 60% through ingesting contaminated food or water. Other studies found that gastrointestinal absorption of Pb varies between 10 to 50% [7], depending on the particle size, lead solubility, nutritional status, and individual activity [8].

The half-life of Pb in bone varies from 15-25 years, and since 90% of its total content accumulates into bones in adults, it can lead to osteoporosis and delay bone fracture healing [9]. According to the evidence, Pb exposure has been linked with increased cancer risk in humans; hence, Pb and its compounds have been known as human carcinogens. Various mechanisms including inhibiting DNA synthesis or regeneration, increasing reactive oxygen reinduction oxidative DNA damage, and enhancing misplaced gene expression, have been proposed to explain lead-induced carcinogenicities [10].

Cd is another heavy metal with toxic properties, which is widely used in the industry along with its components, especially in the battery industry, with an average of 75% [11, 12]. Cadmium-induced environmental pollution occurs through mines, smelters, and cadmium-polluted soils. In the latter case, evidence of contamination of cereals and vegetables with high levels of Cd was reported [13]. Since many plants absorb and accumulate Cd from the soil through the roots, the most critical way humans are exposed to Cd is through feeding. In addition, another form of human exposure to Cd is through air pollution caused by industrial activities. Also, smoking is recognized as a principal non-occupational source of human exposure to Cd [12].

Numerous studies have investigated cadmium-induced toxicity in humans and animals. The role of Cd in lung, kidney, prostate, and bladder cancers and renal, hepatic, sexual, and bone diseases has been demonstrated. Based on the evidence, the half-life of Cd in various tissues has been reported to be about 20 years (14). Some studies have suggested that Cd may have adverse effects on the nervous system of humans and animals. Some studies have also revealed a significant direct relationship between exposure to Cd and abnormal behaviors or intelligence damage in children and adults [12].

Various methods have been proposed to identify metal ion shapes. The polarography method has been introduced as one of the most common methods. In general, polarography is a type of voltammetry where the working electrode is a dropping mercury electrode (DME) to monitor changes in current upon the application of a voltage. In this technique, oxidation and reduction of the current flowing from a cell occurs based on the working electrode potential. Basically, metal ions such as Pb and Cd ions are reduced inside the polarography cell by applying potential and dissolved inside the mercury. The reasons for the superiority of this technique over other methods can be attributed to the wide range of traceable concentrations and measurable inorganic and organic substances and organic metals on the ppb scale. Some advantages of this method over many other common techniques include more comprehensive linear ranges and analysis of a variety of soluble and solid samples. Other advantages of the polarography technique include simultaneous measuring several metals in a sample, decreasing analytical costs compared to the atomic absorption method, and the simplicity of the operation. However, adding automatic sampling systems to this device for industrial uses in quality control is still a severe limit of the routine large-scale uses [15].

Liver is the main place for accumulating toxic compounds, e.g., heavy metals. In this regard, pollution of the environment and pastures with heavy metals originating from various sources lead to the accumulation of these metals in the liver tissue of livestock. Hence, consuming livestock liver is one of the main ways for heavy metals, such as Pb and Cd, to enter the human body through digestion [16]. Simultaneous measuring of Pb and Cd in the same sample, decreasing analytical costs compared to the other methods, and the simplicity of the operation are the novelty of the following study. Therefore, the present study aimed to evaluate the levels of Pb and Cd simultaneously in the liver of sheep in Zanjan and Sanandaj cities, Iran in the second half of July 2014 for the first time, using polarography as a simple and fast method.

2. Materials and Methods

2.1. Sample Preparation

92 samples were randomly prepared in the second half of July 2014 from the central parts of the sheep's liver slaughtered in the slaughterhouses of Zanjan and Sanandaj cities, with 46 samples from each city. Each sample was placed in a numbered transparent zipper food storage bag, transported to the Zanjan School of pharmacy, food and drug control laboratory in iceboxes, and stored at -20 °C until evaluation. ethical committee of the Research Deputy of Zanjan University of Medical Sciences, Zanjan, Iran, approved the study, before sampling and analysis.

2.2. 3.2 Chemicals and Solutions

In this technique, high-purity solvents were used. 65% nitric acid (CAS Number 100456), 30% hydrogen peroxide (CAS Number 107209), mercury with a purity of 99.99% (CAS Number 104403), 3 M sodium hydroxide ultra-pure solution (CAS Number 109137), 1.5 mol/L potassium chloride solution (CAS Number 104938) with 99.99% purity, ready-to-use solution of potassium chloride-sodium acetate (CAS Number 104820) with concentrations of KCl= 1.5 mol/L and CH3COONa= 0.5 mol/L, and standard solutions of 1 g/L of cadmium (CAS Number 119777) and lead (CAS Number 119776), were purchased from Merck, Darmstadt, Germany. It should be noted that deionized water was used in this study.

2.3. Stock Solution Preparation

To prepare stock solutions of the desired metals, 2.5 mL of Cd standard solution (1g/L) and 5 ml of Pb standard solution (1g/L) were added to separate 500 mL volumetric flasks, diluted up to 500 ml with deionized water.

2.4. Samples Digestion

After adapting the samples to lab temperature, 1 g of each liver sample was added to a vessel of a 400-watt microwave digester (SINEO MD-10, Shanghai, China). According to the protocol of the digester for digesting meat products, 6 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide were added to the sample vessel. The samples were digested in 3 steps, including 10 min at 130 °C, 5 min at 150 °C, and 10 min at 180 °C [17]. The digested samples were transferred to Falcon tubes after labeling and kept in the refrigerator at 4 °C until the experiment.

2.5. Analysis

A polarographic system (Model 797 VA, Metrohm, Herisau, Switzerland) equipped with a working electrode (MME; model 6/1246/020, Metrohm, Herisau, Switzerland), reference electrode (Ag/AgCl; model 6/0728/020, Metrohm, Herisau, Switzerland), auxiliary electrode (platinum rod; model 60003/343,000, Metrohm, Herisau, Switzerland), nitrogen (99.99%) pure gas, nitrogen regulator with outlet pressure rating, and mercury with a purity of 99.99% was employed. The adjusted standard method, DIN38406-16, was used for measuring Pb and Cd in digested liquid samples by polarograph. The device was adjusted in the setting and measurement of Pb and Cd [18].

The digested liver samples were adapted to lab temperature. The nitrogen gas pressure was set equal to 1 bar at the outlet gate. The ultra-pure solution of KCl was adjusted to the top of the marker line of the reference electrode. The polarographic cell was washed with deionized water, and 1 mL of digested liver sample was added to the cell. Then, 1.5 mL of potassium chloride-sodium acetate solution and 5mL of deionized water were added to the samples. The pH of the solution was also measured and set by a pH meter (Model 86502, AZ, Taichung Taiwan), and a few drops of ultra-pure 3 M sodium hydroxide solution were added to cell contents for adjusting pH to 4.6 ± 0.2. Eventually, the volume of each cell was then diluted to 11mL with deionized water.

Before measuring the samples, nitrogen gas was blown into the samples to purge the cell contents for 300 s to prevent problems related to the oxygen gas present in the samples. Pb and Cd concentrations in the sample were measured with two duplicates. Since this method uses internal standards for calibration, 0.1 mL of standard solutions of Pb and Cd (with concentrations of 0.5 and 0.1 mg/L) was added simultaneously to the cell containing the sample, and the above steps were repeated. For the second time, standard solutions were added to the polarography cells, and the measurements were repeated. Also, polarograms were drawn during each assay, in which vertical and horizontal axes represented the current intensity and potential difference, respectively. The peak for each metal appeared at a specific potential, which was -0.42 and -0.58 V for Pb and Cd, respectively. The device eventually presented reports following each experiment, and the Pb and Cd concentrations were determined in µg/L [19]. Table 1 shows the parameters used for measuring Pb and Cd by the device.

Table 1: Device Parameters for Measuring the Cd and Pb

Device /parameters	Property/values
Working electrode	HMDE (Hanging Mercury Drop Electrode)
Stirrer speed	2000 rpm (round per minute)
Mode	DP (Differential Pulse)
Purge time	300 s
Deposition potential	-1.15 V
Deposition time	90 s
Equilibration time	10 s
Start potential	-1.15 V
End potential	0.1 V
Pulse amplitude	0.05 V
Pulse time	0.04 s
Voltage step	0.006 V
Voltage step time	0.1 s
Sweep rate	0.06 V/s
Peak potential lead	-0.42 V
Peak potential cadmium	-0.58 V

Briefly, the voltammogram of the digested sample was first measured and recorded. Then, certain volumes of the standard solution were added to the analytes in two steps, and the peak height was calculated based on the obtained voltammograms. Finally, the sample's peak height and standard additions were determined, and values at the intersection of the peak height axis with the concentration axis represented the sample concentration [20].

2.6. Quality Controls of the Method

In this study, values of the limit of detection (LOD) and the limit of quantification (LOQ) were also determined for Pb and Cd metals to evaluate the validation of the test method with suitable levels of accuracy and precision. To calculate the LOD in terms of analytic concentration, a signal equal to the blank signal (yB) was utilized, plus three standard deviations (sB) of the blank signal, (yB+3sB). Additionally, the LOQ was considered the lowest limit for precise quantitative measurements instead of qualitative detection. In order to achieve this limit, a value of yB+10sB has been used [21].

In addition, standard concentrations of 25, 50, 100, and 200 µg/L were prepared from the standard solutions of Pb and Cd to evaluate the accuracy and precision of the method and their values measured by the device. Ultimately, the resulting relative standard deviations (RSDs) were investigated.

2.7. Statistical Analysis

The present study was performed as a descriptive study. Cd and Pb levels were measured, and the results of the polarograph were analyzed by SPSS 18 software (IBM Corp., Armonk, NY, USA). Since the data related to Pb and Cd concentrations in the samples of Zanjan and Sanandaj did not have a normal distribution, the Mann-Whitney U test was employed ($P < 0.05$) to compare the mean concentrations of heavy elements studied in experimental liver samples in each city.

3. Results and Discussion

3.1. LOD, LOQ, Accuracy, and Precision

The signal obtained from 10 blank injections into the polarograph at specific potentials for Cd and Pb were obtained equal to yB= 0.0027 (nA) and yB= 0.0020 (nA), respectively.

In Cd calibration curve, the regression coefficient was 0.999, and the slope of the graph was 32.01. The LOD and LOQ values were equal to 0.253 and 0.843 ppb, respectively. The calibration curve values, including the regression coefficient and the slope of the line for Pb were 0.999 and 21.582. The LOD and LOQ values were equal to 0.208 and 0.926 ppb, respectively.

Cd and Pb evaluation of the liver samples based on LOD and LOQ showed that the percentage of values that are in the detectable and quantifiable range for Cd in Sanandaj and Zanjan cities were 63% and 58%, while for Pb, these values were 50% and 60%, respectively.

Table 2 shows the RSD values reported by the device. The values are close to the actual values, which indicates the accuracy and precision of the reported values of the studied metals in the liver samples.

3.2. Frequency Distribution of Lead and Cadmium in Liver Samples

The frequency distributions of Cd and Pb in the digested liver samples slaughtered in Zanjan and Sanandaj cities are shown in Figures 1 and 2. The results showed that values less than 0.1 mg/kg for Cd and less than 1 mg/kg for Pb had the highest frequency.

3.3. Mean Concentrations of Cd and Pb in Liver Samples

Descriptive statistics of both Cd and Pb metals in samples collected from Sanandaj and Zanjan are presented in Table 3. The obtained results of the Mann-Whitney test showed that there was a significant difference between the mean values of Pb concentrations in the liver samples ($P = 0.0069$), so that the mean levels of Pb in the samples were estimated equal to 3.69 ± 1.43 and 2.36 ± 2.36 mg/kg, respectively.

The values presented in the MRLs standard lists and the WHO standards for Pb in sheep liver samples have been reported 0.5 and 0.31 mg/kg wet weight, respectively. Accordingly, our findings showed that Pb concentrations in the liver samples of sheep slaughtered in Zanjan and Sanandaj were higher than the values of MRLs and WHO standards [5, 22].

Table 2: The Accuracy of the Studied Method Using the Values Prepared from the Standard Solution for Cd and Pb

Parameter	Cadmium (ppb1)	Lead (ppb)
Actual value	200	200
Measured value (Mean ± SD[2])	196.47 ± 7.94	201.04 ± 9.2
RSD[3]	4.04	4.47
Actual value	100	100
Measured value (Mean ± SD)	94.18 ± 1.26	91.25 ± 1.01
RSD	1.33	1.10
Actual value	50	50
Measured value (Mean ± SD)	57.05 ± 0.31	53.15 ± 1.25
RSD	0.54	2.35
Actual value	25	25
Measured value (Mean ± SD)	57.05± 0.31	53.15 ±1.25
RSD	0.54	2.35
Actual value	25	25
Measured value (Mean ± SD)	28.19 ± 0.81	26.39 ± 0.28
RSD	2.87	1.06

ppb1: parts per billion; SD2: standard deviation; RSD3: relative standard deviation

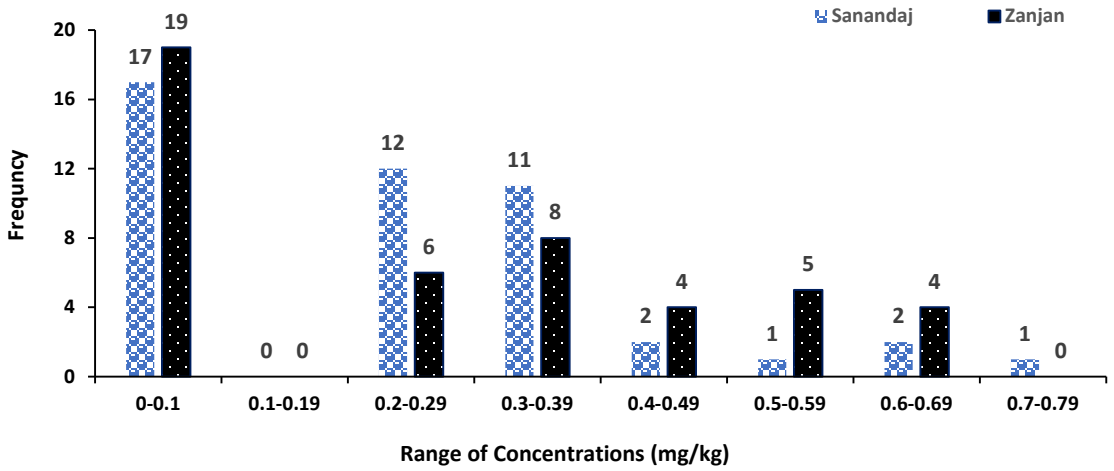

Figure. 1: The Distribution of Cd in the Liver Samples

In contrast, the mean levels of Cd accumulated in the samples (P= 0.8457) had no significant difference. In general, mean levels of Cd in slaughtered sheep were estimated 0.42 ± 0.13 and 0.35 ± 0.12 mg/kg, respectively. The values of MRLs and the WHO standards for Cd in sheep liver samples were equal to 0.5 and 0.2 mg/kg, wet weight, respectively. Results showed that although Cd levels in the samples were higher than WHO standards, they were classified in acceptable ranges of the values presented by MRLs.

Results related to the descriptive comparison of Pb and Cd metals in Sanandaj and Zanjan sheep liver samples (Figures 3 and 4) showed that Pb and Cd levels in Zanjan samples had a higher mean and frequency dispersion than samples collected from Sanandaj city.

Heavy metals-induced environmental pollution is increasingly becoming a problem due to the adverse effects on animal source foods; therefore, consuming these products continuously increases human exposure to heavy metals [23].

In order to control the amounts of residual pollutants in foodstuffs, it is necessary to compile the maximum allowable values of Pb and Cd in different foods. Except for exports, our country complies with international standards such as the Codex Alimentarius Commission (CAC) [24]. According to the evidence, most reports related to detecting heavy metals in food in Iran were related to the fish contamination and other seafood. However, limited reports examined the toxicity of heavy metals, especially Pb and Cd, in beef and its offal. They showed that their Pb levels were lower than the EU standards [4,5].

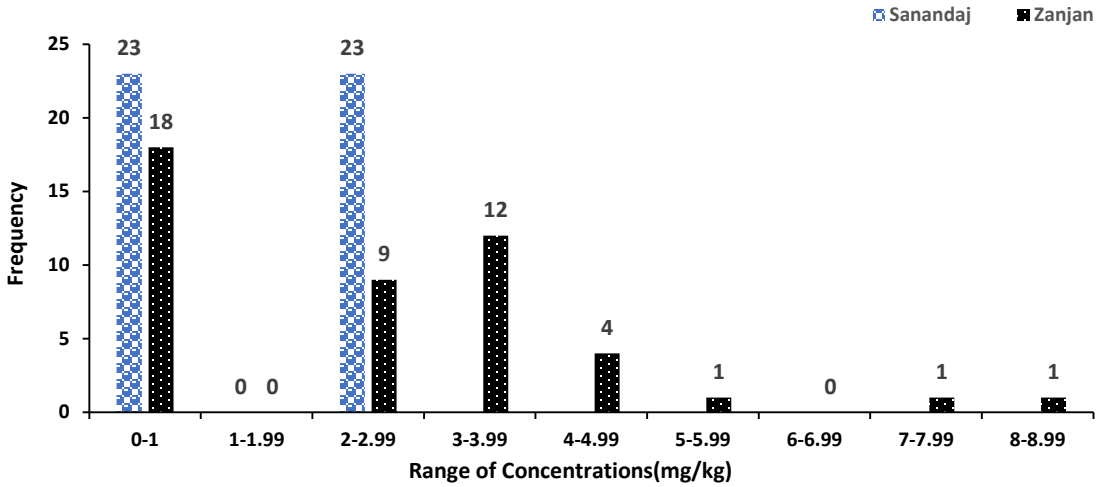

Figure. 2: The Distribution of Pb in the Liver Samples

Table 3: Descriptive Statistics of Pb and Cd in Zanjan and Sanandaj Sheep Liver

Descriptive Statistics Parameters	Lead Values (mg/kg)		Cadmium Values (mg/kg)	
	Zanjan	Sanandaj	Zanjan	Sanandaj
Mean	3.698	2.363	0.421	0.355
Standard Error	0.271	0.038	0.026	0.024
Median	3.375	2.287	0.361	0.308
Mode	N/A*	2.280	0.560	0.310
Standard Deviation	1.435	0.181	0.135	0.130
Sample Variance	2.060	0.033	0.018	0.017
Kurtosis	4.516	-0.749	-1.073	3.153
Skewness	1.969	0.536	0.577	1.908
Range	6.117	0.628	0.427	0.527
Minimum	2.101	2.108	0.263	0.233
Maximum	8.218	2.736	0.690	0.760
Count (No.)	28.000	23.000	27.000	29.000

*N/A: Not Available

The concentrations of Pb accumulated in samples of Zanjan and Sanandaj cities were higher than the thresholds reported by the EU and WHO [5,22]. Rahimi *et al.* (2008) evaluated Pb residue in sheep liver slaughtered at Falavarjan abattoir, Isfahan using graphite furnaces atomic absorption spectrometry equal to 0.18 mg/kg, which was lower than the maximum acceptable threshold determined by the EU [25]. Also, Pb level in the liver of slaughtered cattle in Shahrekord, Iran was estimated 0.245 mg/kg using graphite furnace atomic absorption spectrometry, which was lower than the maximum acceptable threshold established by the European Commission (0.5 mg/kg)[26]. Mansouri *et al.* (2014) also estimated the bioaccumulation level of Pb in the muscle tissue of slaughtered cows in Sanandaj city as 15.5 µg/kg using atomic absorption spectrophotometry, which indicated high contamination levels in these samples [26].Moreover, Zeinali *et al.* (2019) evaluated Pb residue in sheep liver slaughtered at Birjand, Southeast Iran, using an inductively coupled plasma-optic emission spectroscopy (ICP-OES) equal to 0.085 ± 0.03 mg/kg, which was lower than the maximum acceptable threshold determined by the European Union [28].

Our findings also estimated the mean Cd levels in Zanjan and Sanandaj sheep liver samples were lower than the threshold advised by the EU (0.5 mg/kg) and above the standard announced by the WHO (0.2mg/kg). In this regard, the level of Cd accumulated in the sheep liver collected from Shahrekord, Iran was equal to 0.02 mg/kg [25], and the bioaccumulation of Cd in the muscle tissue of cows slaughtered in the Sanandaj city was equal to 0.01 mg/kg [25]. Zeinali *et al.* (2019) evaluated cadmium residue in the sheep liver of Birjand (Iran) using an inductively coupled plasma-optic emission spectroscopy (ICP-OES) equal to 0.025 ± 0.01mg/kg, which was lower than the maximum acceptable threshold determined by the EU [28].

As mentioned, limited studies have been conducted in Iran to determine the concentration of heavy metals in the livestock liver, especially sheep. On the other hand, it may not be logical to compare results reported by other researchers in other countries with our findings due to spatial differences, measurement methods, livestock type, and tissue type. However, similar studies have been conducted in other countries. Massadeh *et al.* (2006) evaluated the levels of Pb accumulated in local Jordanian and imported Australian sheep livers using atomic absorption spectrometry and showed that Pb concentrations in the liver of local and imported sheep were equal to 0.004 and 0.005 mg/kg, respectively [29]. Akoto *et al.* (2014) also examined the distribution of Pb and Cd concentrations in Ghana sheep liver using atomic absorption spectrometry. They calculated the concentrations of accumulated Pb and Cd equal to 0.14 and 0.07 mg/kg, respectively [30]. MacLachlan *et al.* (2016) evaluated Pb and Cd concentrations in liver, kidney, and muscle in Australian sheep by inductively coupled optical emission spectroscopy (ICP-OES). Mean levels found in muscle, livers, and kidneys were 0.0035, 0.280 and 0.853 mg/kg for Cd and 0.007, 0.040 and 0.057 mg/kg for Pb; mg/kg, respectively. These values are lower than the maximum acceptable threshold determined by the EU [31].

Elevated levels of Pb and Cd in liver samples collected from Zanjan compared to Sanandaj and WHO standards can be attributed to the activities of industries and lead, copper, and zinc extraction factories in this city, which leads to pollution of surface and groundwater resources, air, and pastures used by ranchers and villagers. Also, active metal mining mines surrounding Sanandaj city can be considered one of the main reasons for the elevated Pb in liver samples collected from this city. However, further studies are required to determine the role of these industries and factories.

According to the above statements, increasing knowledge and understanding is essential to prevent and control the origin and possibilities of food contamination by various methods. Duo to limited studies have been conducted on the level and method of contamination of water resources, fodder/animal feed, and various livestock products with heavy metals, further and continuous monitoring of the quality of relevant resources should be a priority for food hygiene in Iran.

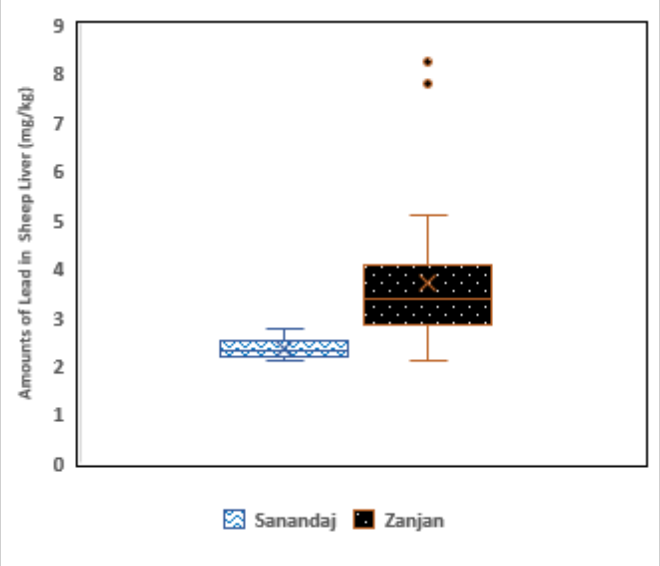

Figure 3: Boxplot and comparison of means of Pb concentration in liver samples collected from Zanjan and Sanandaj cities

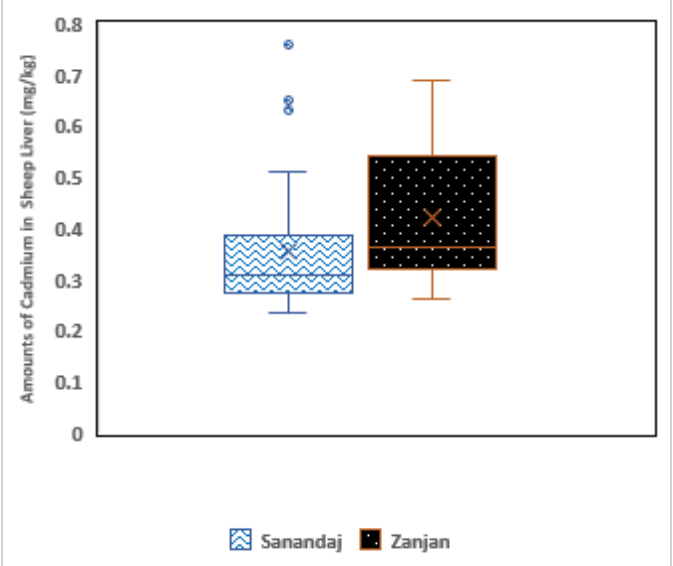

Figure 4: Boxplot and comparison of means of Cd concentration in liver samples collected from Zanjan and Sanandaj cities

4. Conclusion

In conclusion, our findings showed that a significant difference was observed only between levels of Pb in Zanjan and Sanandaj sheep liver, and the concentrations of Pb and Cd in both samples were higher than the WHO standards. This study and other similar internal studies indicated that some animal source foods, such as sheep liver and other meat products, are contaminated with heavy metals. Hence, the presence of these foods in people's food baskets and their consumption by some members of society may increase the risks and complications induced by the accumulation of these metals in the body of children, pregnant and lactating mothers, and the elderly.

Authors' Contributions

Saman Skandari: Investigation, Data curation, Resources, Writing – original draft. **Saeed Rezaee:** Formal analysis, Supervision, Validation. Project administration. **Farideh Dinmohammadi:** Resources, Investigation, Data curation, Methodology. **Mehran Mohseni:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review and Editing.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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