Determination of some heavy metals concentration in species animal meat (sheep, beef, turkey, and ostrich) and carcinogenic health risk assessment in Kurdistan province, western Iran

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Research Article

Keywords: Environmental Pollution, Heavy metal, Target Risk of cancer, Meat, Sanandaj-Iran

Posted Date: May 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-407310/v1

Abstract

Accumulation of heavy metals in food as one of the environmental pollutants due to the development of urban industries and human activities, is one of the threats to public health. The purpose of this study, as the first report in Sanandaj, one of the strategic cities of western Iran, was to investigate the concentration of heavy metals in the meat of different species of sheep, calves, turkeys and ostriches. In a cross-sectional study, the content of selenium, lead, cadmium, arsenic, cobalt, zinc, nickel, copper, and chromium in 170 meat samples prepared from meat distribution centers in Sanandaj was investigated. The content of these elements was measured by the ICP-MS method in the meat of beef, sheep, turkey, and ostrich. Data were analyzed using parametric tests of ANOVA and one-sample t-test, and the correlation coefficient and the relationship among the concentration of metals were also calculated and compared in studied species. The results showed that the mean content of selenium, nickel, cobalt, and chromium in the studied species did not show a significant difference ($P > 0.05$). However, the mean content of lead, cadmium, arsenic, zinc, and copper showed a significant difference ($P < 0.05$). A significant negative correlation was also found between cadmium and selenium in turkey meat. With the exception of copper and selenium, the concentrations of other metals were higher than allowed in the meat of the studied species. The Target Risk (TR) of cancer for lead, cadmium, and arsenic in some species studied was higher than allowed (more than 10^{-4}). Due to the excessive accumulation of carcinogenic heavy metals in sheep, beef, turkey, and ostrich meat, it is necessary to monitor and remove barriers to environmental pollutants.

Introduction

Based on food safety, achieving sufficient healthy and safe food is one of the most important pillars of community health, and unsafe food is a threat to global health and endangers everyone. Harmful bacteria, viruses, parasites, or chemicals are important threats to food health, causing a wide range of diseases from diarrhea to cancer(World Health 1999). Approximately 1 in 10 people in the world become ill after eating contaminated food. About 420,000 people die each year from unsafe food, and children under the age of 5 make up 40% of it with 125,000 deaths(Fukuda 2015; World Health 1999). Infants, young children, the elderly, pregnant women, and those with underlying diseases are most susceptible to the disease. Insecure food and food-borne diseases(Wagstaff 2002), in addition to the high cost of medicine and health care, damage national economies, tourism, and trade, and delay socio-economic development(Braveman and Gruskin 2003). Foodborne diseases are caused by bacteria, viruses, or chemicals that enter the body. Foodborne illness can be caused by the toxic nature of food, or the presence of bacteria, viruses or chemicals in it. Persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), and heavy metals are among the chemical factors that cause food contamination and their long-term exposure can affect the immune system and natural growth and causes cancer(Bintsis 2017; Kuchenmüller et al. 2013). Heavy metals are any metal that is potentially toxic and has a specific gravity greater than 5.0. Vanadium (V), cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), iron (Fe), cobalt (Co), manganese (Mn), and zinc (Zn) are the most important(Jaishankar et al. 2014).

Heavy metals are generally divided into three groups: essential metals (copper, zinc, iron), possibly essential metals such as cobalt and vanadium, and non-essential or toxic metals such as mercury, lead, and cadmium(Raikwar et al. 2008). There is an optimal concentration for essential metals and possibly essential metals for normal body chemical processes, and non-essential or toxic metals are toxic in any amount. These metals can accumulate in the tissues of the human body, animals, and plants, and their main problem is that they are not metabolized by biological systems(Jaishankar et al. 2014; Raikwar et al. 2008). Contamination by heavy metal in food occurs mainly through pollution of air, water, and soil as well as through livestock and plant products grown on pollution-prone farms(Singh et al. 2011). Heavy metals as pervasive and mobile pollutants can be easily available from soil, fertilizers, air, industrial processes, and transportation(Jaishankar et al. 2014). Due to the presence of many metals in food as natural components of foodstuffs as well as contamination during processing and environmental contamination, consumption of food is the main source of exposure to heavy metals(Singh et al. 2011). However, contamination of meat and livestock products with heavy metals does not occur during processing, but the main cause of contamination is feeding livestock and poultry with contaminated food or raising them near contaminated areas(Singh et al. 2011). Therefore, there are significant concerns about the prevalence of heavy metals as a potentially toxic and their adverse effects on public health. One of the threats that can endanger the quality of human life is the high levels of these metals in foods. Food safety, nutrition, and food security are inextricably linked and are one of the criteria for preparing, storing and selecting food that is affected by drug and heavy metals residues(Rai et al. 2019). Therefore, the study of heavy metal concentrations in food is of great biological importance, because the assessment and control of food contamination and the identification of contaminants, their modification or elimination will have a significant impact on human health and longevity(Hu et al. 2018; Jaishankar et al. 2014). It has been widely reported that contamination of meat and other livestock products with heavy metals is also a concern and threatens food hygiene and human health because these metals are not naturally present in edible tissues and can cause severe side effects even in very small amounts(Sardar et al. 2013). These metals cause residual sediment in livestock meat as a very rich and available source of nutrients. Although acute exposure to these elements through food can rarely cause poisoning, their accumulation in the body leads to adverse effects on human health. Beef, mutton, chicken, and turkey are used as the most widely consumed food in Iran and many countries due to easy access and valuable nutrients such as protein, vitamins, and essential minerals. Protein products origin from beef, mutton, and chicken are one of the basic and essential nutritional needs of humans, which are rich sources of minerals and amino acids, and this shows their nutritional value for being in the food chain(Ahmad et al. 2018).

In recent years, the importance of paying attention to the issue of food poisoning with heavy metals has increased due to the increasing number of food-borne diseases(Elsharawy and Elsharawy ; Rather et al. 2017). Studies show that human exposure to heavy metals causes adverse effects, such as developmental retardation, several types of cancer, kidney damage, endocrine disruption, immunological disorders and even death. Metal poisoning can include damage to or disruption of the cardiovascular system, detoxification pathways (liver and kidneys), endocrine hormone glands, energy pathways, enzymes, digestive system, immune system, central nerves, reproduction, and urinary tract(Mamtani et al. 2011). Heavy metals, including lead, mercury, and cadmium, have unknown functions despite being toxic and increase oxidation once they enter the human body(Rather et al. 2017). Oxidation itself is one of the pathomechanisms of diseases such as hypertension and diabetes. Several epidemiological studies have confirmed the effects of heavy metals, including lead, mercury, and cadmium, in the development of metabolic and cardiovascular diseases(Alissa and Ferns 2011; Jennrich 2013). As mentioned, some heavy metals are essential elements for humans, animals, and plants, and although small amounts of these metals are essential in many of the body's reactions and activities, they cause more or more irreversible side effects(Engwa et al. 2019). Therefore, one of the most important things, to issue a food hygiene certificate, is to check the amounts of these metals in animal and plant products. Due to the pollution of the environment with industrial and non-industrial wastes as well as the type of feeding and breeding of livestock, the possibility of pollution and the presence of heavy metals in this valuable food source is not far from expectation and therefore it may be caused health problems for consumers of this food source in the future. In addition, the presence of heavy metals as one of the disruptors of health and environmental pollution has been mentioned in developed or developing industrial societies, therefore, the study of health and safety of food of animal origin in Iran as a developing country have great importance(Ali et al. 2019). The aim of this study was to investigate the concentration of heavy elements such as lead (Pb), cadmium (Cd), Arsenic (As), zinc (Zn), nickel (Ni), copper (Cu), chromium (Cr), cobalt (Co) and selenium (Se) in distributed sheep, beef, turkey and ostrich meats in Sanandaj city, Iran.

Materials And Methods

This cross-sectional study was conducted in 2020 in Sanandaj, Iran. The number of meat supply centers in the city of Sanandaj is 170 centers, of which 50%, i.e. about 80 centers are located in the center of the city. The rest of these numbers are scattered in four geographical areas: north, south, east, and west. Due to the high dispersion, there are no accurate statistics on the percentage of dispersion in these four areas. This statistic is documented in the veterinary network of Sanandaj. 45 samples of beef, 45 samples of sheep, 40 samples of turkey, and 40 samples of ostrich were randomly collected from distribution centers in this city.

All solutions were prepared from analytical grade reagents. Double distilled water applied throughout this study was ultrapure quality. Nitric acid and hydrogen peroxide with supra pure quality obtained from Merck (Darmstadt, Germany). Standard solutions of Cr, Ca, and Pb with appropriate dilution (1000 mg/ml) were purchased from Sigma (Chem. Co., St. Louis, USA).

10 g of each sample was sampled with a sterile surgical blade. The samples were immediately placed on dry ice in sterile containers and immediately sealed to maintain a cold chain. The samples were then transferred to the laboratory to prepare for the measurements. These were processed for analysis within one week of their purchase, well before the expiry date. After this time, the samples were first dried, crushed, and then ground and 25 g of powder of each sample was used for measurement. Metal concentrations were determined based on the dry weight of the samples.

In this study to measure the level of heavy metal in our sample inductively coupled plasma mass spectrometry (ICP-MS) was used. ICP-MS is a type of mass spectrometry that uses an inductively coupled plasma to ionize the sample. It atomizes the sample and creates atomic and small polyatomic ions, which are then detected. It is known and used for its ability to detect metals and several non-metals in liquid samples at very low concentrations. It can detect different isotopes of the same element, which makes it a versatile tool in Isotopic labeling. Compared to atomic absorption spectroscopy, ICP-MS has greater speed, precision, and sensitivity.

Pyrex glass digestion tubes (Foss, USA) were used for digestion. Each tube was washed with a solution of 10% nitric acid for 48 h followed by rinsing with double distilled water to decrease external metal contamination. 0.2g of each sample was digested with 3 mL of nitric acid (70%, Merck) and 1.0 mL of hydrogen peroxide (H2O2) (30%) for 8 h at 160 ˚C until the tissue was completely dissolved. The temperature was increased gradually, starting from 50 C and increasing up to 160 C. After cooling, the digested solution was filtered through Whatman filter paper No. 1. The mixture subsequently was poured into the labeled containers and diluted to 10 ml with double distilled water and used for analysis by a SCIEX ELAN DRC II ICP-MS (PerkinElmer). Analyzing heavy metals concentrations (Cd, Cr, and Pb) in samples was carried out by graphite furnace atomic absorption spectrometry (Varian model spect, AA 240, USA) using high purity argon as the inert gas. For the quantitative analysis of the samples, an external calibration technique was followed. All metals were calibrated using a multi-element standard (IV-ICPMS-71A, Inorganic Ventures), diluted to a 0.05 μg/mL concentration. Standard solutions were prepared in 19.6% (w/w) HNO3 (the same percentage of acid present in the samples) by diluting a multi-element standard solution containing all the elements. The calibration curves for all the analytes were built on six different concentrations, from the limit of detection (LOD) of the corresponding element up to 200 ng/g. All the measurements were carried out using the full quantitative mode analysis. The correlation coefficients for all the calibration curves were at least 0.9999, showing good linear relationship throughout the ranges of concentrations studied. The absence of polyatomic interferences was checked by measuring several isotopes of the elements and checking the isotopic ratio in the digested solution of the samples.

A total of three replicates were measured from each sample, and a set of blank control (reagents only) replicates were measured to correct for contamination during processing.

Statistical analysis

Data were analyzed using SPSS software version 23 and reported as mean ± standard deviation (SD), minimum and maximum for each group (livestock type). The normality of data distribution was checked using the Kolmogorov-Smirnov test. Pearson correlation test was used to determine the correlation between the concentrations of heavy metals lead, cadmium, and arsenic with selenium by type of meat. One-way analysis of variance (ANOVA) was used to compare the mean of concentrations of lead, cadmium, arsenic and selenium among groups of distributed meat (livestock type). Due to the high importance of toxicity of lead, cadmium, and arsenic, a one-sample t-test was used to evaluate and compare the concentration of arsenic, lead, and cadmium with the permissible consumption in the meat tissue of the studied animals. The significance level in this study was considered less than $p < 0.05$.

Results

The results showed that there was a difference between the accumulation of metals in different species

In all studied species, the mean ± SD of the metals was as the following: selenium 1.22 ± 0.56, lead 4.76 ± 0.41, cadmium 1.89 ± 0.43, arsenic 0.54 ± 0.036, zinc 198.41 ± 71.63, nickel 0.75 ± 0.081, cobalt 0.068 ± 0.004, copper 10.1 ± 5.66 and chromium was 2.11 ± 1.3 mg/kg. The results of the Kolmogorov-Smirnov test showed that the mean of accumulated concentrations of the evaluated elements in all studied species had a normal distribution. The highest amount of lead accumulation was in sheep (11.79 ± 4.217), and beef (7.01 ± 2.718) mg/kg, and the lowest amount was in turkey (0.13 ± 0.02) and ostrich (0.09 ± 0.01) respectively. Therefore, according to the average accumulated concentration of lead, the general pattern of accumulation of this element in the studied species was sheep > beef > turkey > ostrich, respectively (Fig. 1- A). There was also a significant difference among the four species in terms of lead level ($F = 3.610$, $df = 3$, $P = 0.022$).

Cadmium levels in the studied species included beef (4.31 ± 2.89), sheep (2.80 ± 1.98), turkey (0.29 ± 0.13), and ostrich (0.16 ± 0.05), respectively. Therefore, considering the average accumulated concentration of cadmium, the general pattern of accumulation of this element in the studied species was beef > sheep > turkey > ostrich. According to the obtained differences, the type of species affected the accumulation of cadmium ($F = 13.08$, $df = 3$, $P < 0.001$) (Fig. 1-B).

The results showed that the arsenic content in the studied species was turkey (0.88 ± 0.21), ostrich (0.85 ± 0.13), beef (0.23 ± 0.14) and sheep (0.20 ± 0.09). The results of arsenic analysis in the studied species showed that the highest rate of accumulation of this metal was in turkey and then in ostrich and the lowest in beef and then in sheep. Therefore, considering the mean accumulated concentration of arsenic, the general pattern of accumulation of this element in the studied species was turkey > ostrich > calf > sheep (Fig. 1-C). The results showed that there was a significant difference among the four types of species in terms of arsenic mean accumulation ($F = 56.48$, $df = 3$, $P < 0.001$).

The results of selenium content analysis in the studied species showed that the highest content of this metal was observed in beef (1.37 ± 0.74), sheep (1.33 ± 0.79), then turkey (1.15 ± 0.27), and the lowest was in ostrich (1.02 ± 0.08). Therefore, the general pattern of accumulation of this element in the studied species can be beef > sheep > turkey > ostrich, respectively (Fig. 2). However, the results showed that selenium content had no significant difference among these four species ($F = 0.773$, $df = 0.3$, $P = 0.571$) (Fig. 2-D).

The highest level of zinc was also in beef (234.60 ± 63.48), sheep (233.10 ± 51.82) and then ostrich (0.85 ± 0.13), and the lowest amount was in turkey (116.20 ± 43.69). Therefore, the general pattern of zinc content in the studied species can be beef > sheep > ostrich > turkey, respectively (Fig. 2-E). The results also showed that there was a significant difference in the mean content of zinc among the four species ($F = 16.49$, $df = 3$, $P < 0.001$).

The results of nickel content analysis in the studied species showed that the highest content of this metal was in beef (1.17 ± 1.44), ostrich (0.65 ± 0.17), and then turkey (0.63 ± 0.28), and the lowest accumulation was in sheep (0.54 ± 0.57). Therefore, the general pattern of accumulation of this element in the studied species can be beef > ostrich > turkey > sheep, respectively. There was no significant difference in the mean content of nickel among the four species ($F = 1.31$, $df = 3$, $P = 0.287$). In other words, the effect of species on the concentration of nickel was insignificant (2-F).

The results of chromium content analysis in the studied species showed that the highest level of this metal was in turkey (2.73 ± 2.17), ostrich (2.10 ± 0.71) and then beef (1.84 ± 0.30), and the lowest level was in sheep (1.66 ± 0.48). Therefore, the general pattern of content of this element in the studied species can be turkey > ostrich > beef > sheep. The results showed that there was no significant difference in the mean of chromium among the four species ($F = 1.51$, $df = 3$, $P = 0.229$). In other words, the effect of species on the concentration of chromium was not significant (Fig. 2- H).

The results of determining the amount of cobalt in the studied species showed that the highest amount of this metal was in turkey (0.077 ± 0.01), sheep (0.075 ± 0.07) and then ostrich (0.074 ± 0.01), and the lowest amount was in beef (0.045 ± 0.01). Therefore, the general pattern of accumulation of this element in the studied species can be turkey > sheep > ostrich > beef, respectively. The results obtained from one-way analysis of variance showed that there was no significant difference between species in the accumulation of this metal ($F = 1.13$, $df = 3$, $P = 0.351$) (2-G). The results showed that the highest amount of copper was in turkey (16.28 ± 4.87), ostrich (11.83 ± 3.63), and then beef (5.75 ± 0.92), and the lowest accumulation was in sheep (5.56 ± 1.19). Therefore, the general pattern of accumulation can be turkey > ostrich > beef > sheep (Fig. 2-K). The results showed that there was a significant difference in the mean of copper level among the four species ($F = 28.15$, $df = 3$, $P < 0.001$).

Table 1
mean ± SD, median, min and max of heavy metals in sheep, beef, turkey and ostrich

Metals	Species	Mean ± SD	Median	Min- Max	F, P-Value
Se (mg/kg)	Sheep	1.32 ± 0.79	1.92	0.31–2.53	F = 0.733, df = 3, p = 0.517
	Beef	1.37 ± 0.23	1.25	0.5–2.57	
	Turkey	1.15 ± 0.27	1.10	0.9–1.81	
	Ostrich	1.02 ± 0.1	0.97	0.94–1.12	
Pb (mg/kg)	Sheep	11.79 ± 17.5	5.93	0.91–58.9	F = 3.6, df = 3, p = 0.022*
	Beef	7.01 ± 7.3	3.14	1.51–21.5	
	Turkey	0.13 ± 0.03	0.13	0.1–0.12	
	Ostrich	0.9 ± 0.1	0.1	0.08–0.11	
Cd (mg/kg)	Sheep	2.79 ± 1.9	2.11	0.75–6.13	F = 13.1, df = 3, p < 0.001*
	Beef	4.31 ± 2.8	3.77	0.47–10.63	
	Turkey	0.29 ± 0.13	0.25	0.190–0.73	
	Ostrich	0.16 ± 0.05	0.18	0.06–0.21	
As (mg/kg)	Sheep	0.23 ± 0.14	0.21	0.06–0.5	56.5, df = 3, p < 0.001*
	Beef	0.2 ± 0.09	0.19	0.7 – 0.33	
	Turkey	0.88 ± 0.21	0.79	0.58–1.28	
	Ostrich	0.85 ± 0.13	0.84	0.68–1.1	
Zn (mg/kg)	Sheep	233.10 ± 51.82	231	145–317	16.5, df = 3, p < 0.001*
	Beef	234.6 ± 63.48	226.5	149–323	
	Turkey	116.20 ± 43.69	111.79	55–178	
	Ostrich	223.1 ± 20.17	225.55	207–268	
Ni (mg/kg)	Sheep	0.54 ± 0.57	0.35	0.02-2	F = 1.31, df = 3, p = 0.287
	Beef	1.17 ± 1.4	0.54	0.11–4.95	
	Turkey	0.63 ± 0.26	0.62	0.32-1	
	Ostrich	0.65 ± 0.17	0.69	0.40–0.88	
Co (mg/kg)	Sheep	0.05 ± 0.04	0.013	0.01–0.11	F = 1.13, df = 3, p = 0.351
	Beef	0.08 ± 0.07	0.053	0.006–0.193	

Note: Data presented as mean ± SD; ***=** significant at p < 0.05, using analysis of variance (ANOVA)

Metals	Species	Mean ± SD	Median	Min- Max	F, P-Value
	Turkey	0.077 ± 0.01	0.073	0.06–0.09	
	Ostrich	0.074 ± 0.01	0.072	0.06–0.08	
Cr (mg/kg)	Sheep	1.66 ± 0.4	1.58	1.02–2.7	F = 1.51, df = 3, p = 0.229
	Beef	1.83 ± 0.3	1.92	1.43–2.18	
	Turkey	2.72 ± 0.1	2.12	0.81–8.8	
	Ostrich	2.1 ± 0.7	1.98	1.18–3.07	
Cu (mg/kg)	Sheep	5.56 ± 1.19	6.25	3.12–6.25	F = 28.15, df-3, p < 0.001*****
	Beef	5.75 ± 0.9	6.25	3.75–6.25	
	Turkey	16.28 ± 4.8	15.89	7.21–23.53	
	Ostrich	11.83 ± 3.63	11.75	6.94–17.24	

Note: Data presented as mean ± SD; ***=** significant at p < 0.05, using analysis of variance (ANOVA)

Results of Pearson correlation analysis after the investigation of the relationship between metal concentrations based on species (sheep, beef, turkey, and ostrich)

The results of Pearson correlation analysis to investigate the relationship among the concentrations of the studied metals with others in species are presented in Tables 2–5. The correlation matrix of the concentrations of the studied metals in the sheep specie is shown in Table 3.

According to Pearson correlation analysis, in sheep samples, there was a significant negative correlation between chromium and copper ($P = 0.010$, $r = 0.764$). Also, no significant correlation was observed among any of the other metals in sheep samples (P-value > 0.05, Table 2).

Table 2

— Correlation Matrix between Metal Concentrations in sheep

	Se	**Pb**	**Cd**	**As**	**Zn**	**Ni**	**Cr**	**Co**	**Cu**
Se	1								
Pb	0.340	1							
Cd	-0.254	-0.323	1						
As	0.136	-0.172	0.423	1					
Zn	-0.088	0.101	-0.003	-0.059	1				
Ni	-0.207	0.217	0.464	0.281	0.157	1			
Co	-0.481	-0.315	0.350	-0.160	-0.159	0.075	1		
Cr	0.212	-0.456	0.092	0.063	-0.311	0.216	-0.487	1	
Cu	0.474	0.131	-0.110	0.053	-0.313	-0.263	-0.764*	-0.148	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

The correlation matrix of the concentrations of the studied metals in the beef specie is shown in Table 3. According to Pearson correlation analysis, in beef samples, there was a negative and significant correlation between chromium and copper (P = 0.002, r = 0.846). However, no significant correlation was observed among any of the others (P-value > 0.05, Table 3).

Table 3
— Correlation Matrix between Metal Concentrations in beef

	Se	Pb	Cd	As	Zn	Ni	Cr	Co	Cu
Se	1								
Pb	0.184	1							
Cd	0.458	0.392	1						
As	-0.059	-0.505	-0.350	1					
Zn	-0.356	0.477	0.033	-0.511	1				
Ni	0.152	-0.102	0.322	-0.032	-0.620	1			
Co	0.367	0.533	0.134	-0.310	-0.145	0.452	1		
Cr	-0.291	-0.476	0.177	0.249	-0.421	0.537	-0.178	1	
Cu	-0.061	-0.507	0.167	0.281	0.108	-0.412	-0.846**	0.257	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

Based on Pearson correlation analysis, in turkey samples, there was a positive and significant correlation between arsenic and zinc (P = 0.009, r = 0.712), arsenic and nickel (P = 0.005, r = 0.751), nickel and zinc (P < 0.001, r = 0.891), copper and arsenic (P = 0.009, r = 0.717), copper and zinc (P = 0.016, r = 0.677), copper and Nickel (P = 0.042, r = 0.593). However, there was a negative and significant correlation between selenium and cadmium (P = 0.007, r = -0.728), lead and arsenic (P = 0.001, r = -0.81), lead and zinc (P = 0.001, r = -0.808), lead and nickel (P = 0.01, r = -0.812) and between lead and copper (P = 0.01, r = 0.815). No significant correlation was observed among other metals (all P-value > 0.05) (Table 4).

Table 4
— Correlation Matrix between Metal Concentrations in Turkey

	Se	Pb	Cd	As	Zn	Ni	Cr	Co	Cu
Se	1								
Pb	-0.366	1							
Cd	-0.728**	0.284	1						
As	0.190	-0.810**	0.253	1					
Zn	0.524	-0.808**	0.396	0.712**	1				
Ni	0.568	-0.812**	0.364	0.751**	0.891**	1			
Co	-0.044	-0.247	-0.091	0.018	0.477	0.323	1		
Cr	-0.305	-0.213	0.114	0.541	0.358	0.322	0.304	1	
Cu	0.213	-0.815**	0.291	0.717**	0.677*	0.593*	0.301	0.238	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

Based on Pearson correlation analysis, in ostrich samples, there was a positive and significant correlation between arsenic and nickel (P = 0.030, r = 0.756), zinc and nickel (P = 0.026, r = 0.768). However, a significant negative correlation was observed between arsenic and selenium (r = -0.83, P = 0.019), lead and cadmium (P = 0.008, r -0.847), copper and selenium (P = 0.003, r = -0.895) and copper and cadmium (P = 0.026, r = -0.770). The correlation of other metals with each other was not significant (Table 5).

Table 5
— Correlation Matrix between Metal Concentrations in Ostrich

	Se	Pb	Cd	As	Zn	Ni	Cr	Co	Cu
Se	1								
Pb	-0.649	1							
Cd	0.666	-0.847**	1						
As	-0. 83	-0.247	0.034	1					
Zn	0.415	-0.621	0.362	0.359	1				
Ni	0.153	-0.394	0.101	0.756*	0.768*	1			
Co	0.573	-0.162	0.158	-0.538	-0.208	-0.343	1		
Cr	0.519	-0.246	-0.128	0.114	0.526	0.428	0.293	1	
Cu	-0.895**	-0.561	-0.770*	0.315	-0.338	0.017	-0.498	-0.188	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

Results of one-sample t-test of comparison of the concentrations of lead, cadmium, arsenic, zinc, nickel, chromium with the permissible limit of consumption in the tissues of sheep, beef, turkey, and ostrich

The comparison of the average concentration of heavy metals with the allowable consumption limit in sheep tissue showed that considering the statistical comparison at 95% confidence level, the average concentration of lead, cadmium, arsenic, chromium, zinc, and copper accumulated in sheep was higher than the maximum allowable consumption limit (P-value < 0.05). But the concentration of nickel and cobalt accumulated in sheep was less than the maximum allowable concentration of standard consumption.

The comparison of the average concentration of heavy metals with the allowable limit of consumption in beef samples showed that considering the statistical comparison at 95% confidence level, the mean concentration of lead, cadmium, arsenic, chromium, zinc, and copper content in beef samples was higher than maximum permissible concentration limit (P < 0.05). Nevertheless, the concentration of nickel and cobalt accumulated in the beef samples was not more than the maximum allowable concentration of consumption limit (P > 0.05).

In turkey, in terms of statistical comparison at 95% confidence level, the mean concentration of lead, arsenic, chromium, nickel, zinc and copper accumulated in turkey samples was more than the maximum allowable concentration (P < 0.05). In contrast, the mean concentration of cadmium was not more than the maximum allowable concentration in turkey samples (P > 0.05).

Based on one-sample t-test, the mean concentrations of cadmium, arsenic, zinc, chromium and nickel accumulated in ostrich samples were higher than the maximum allowable concentration (P-value < 0.05). But the concentrations of lead, cobalt and copper were assessed as permissible (Table 6).

Table 6
Comparison of Metal Concentrations with WHO/FDA standard values using one sample t-test

Parameter	Groups			
	Sheep (MD, (95% CI)	**Beef** (MD, (95% CI)	**Turkey** (MD, (95% CI)	**Ostrich** (MD, (95% CI)
Pb (mg/kg)	11.69(0.82 to 24.21)	6.91(1.62 to 12.21)	0.03(0.01 to 0.04)	-0.002(-0.01 to 0.01)
	P-value = 0. 044*	p-value = 0.016*	p-value = 0.008*	p-value = 0.563
Cd (mg/kg)	2.49(1.1 to 3.9)	4.01(1.93 to 6.1)	-0.01(-0.09 to 0.07)	-0.14(-0.18 to -0.10)
	P-value = 0. 003*	P-value = 0. 002*	P-value = 0.824	P-value = 0. 001*
As (mg/kg)	0.13(0.03 to 0.23)	0.10(0.03 to 0.17)	0.78(0.64 to 0.91)	0.75(0.64 to 0.86)
	p-value = 0.017*	p-value = 0.008*	p-value < 0.001*	p-value < 0.001*
Zn (mg/kg)	203.1(166.1 to 240.2)	204.6(159.2 to 250.1)	86.2(58.44 to 113.96)	203.1(186.2 to 219.9)
	p-value < 0.001*	p-value < 0.001*	p-value < 0.001*	p-value < 0.001*
Ni (mg/kg)	0.16(-0.25 to 0.58)	0.79(-0.24 to 1.83)	0.25(0.07 to 0.41)	0.27(0.12 to 0.41)
	p-value = 0.400	p-value = 0.117	p-value = 0.009*	p-value = 0.003*
Co (mg/kg)	0.003(-0.03 to 0.04)	0.03(-0.02 to 0.08)	0.03(0.03 to 0.04)	0.03(0.02 to 0.03)
	p-value = 0.870	p-value = 0.211	p-value < 0.001*	p-value < 0.001
Cr (mg/kg)	1.46(1.11 to 1.80)	1.64(1.42 to 1.85)	2.52(1.45 to 3.91)	1.90(1.31 to 2.49)
	p-value < 0.001*	p-value < 0.001*	p-value = 0.002*	p-value < 0.001*
Cu (mg/kg)	-4.44(-5.29 to -3.58)	-4.25(-4.91 to -3.59)	6.28(3.19 to 9.38)	1.83(-1.21 to 4.87)
	p-value < 0.001*	p-value < 0.001*	p-value = 0.001*	p-value = 0.197

Note: Data presented as Mean differences (95% CI); *= significant at p < 0.05, using on one sample t-test; WHO/FDA standard values: Pb = 0.1, Cd = 0.3, As = 0.1, Zn = 30, Ni = 0.38, Co = 0.043, Cr = 0.2, Cu = 10

In relation to the non-carcinogenic risk factor of heavy metals, there is a parameter called THQ (target hazard Quotient) which is calculated using the following formula:

$$THQ = (ED \times EF \times IR \times CM \div WAB \times ATn \times RfD) \times 10^{-3}$$

With regard to THQ, values less than 1 are acceptable; Therefore, it can be said that the amount of lead in beef and sheep meat, the amount of arsenic in sheep, turkey and ostrich meat and the amount of cadmium in beef and ostrich meat had higher THQ than the allowable level, which could be a health warning (Table 7).

Table 7: THQ measurements of lead, cadmium and arsenic metals in different species meat studied

As	Cd	Pb	THQ
1.415	0.775	169.006	**Sheep**
0.393	271.629	49.511	**Beef**
1.785	0.53	0.079	**Turkey**
2.015	2.651	0.023	**Ostrich**

To measure the risk of cancer in heavy metals, the TR (Target Risk of cancer) parameter is used, which is calculated according to the following formula:

$$TR = (ED \times EF \times IR \times CM \times CPSo \div WAB \times TAc) \times 10^{-3}$$

TR has less than 10^{-6} food safety. Between 10^{-6} and 10^{-4} is considered satisfactory. More than 10^{-4} has cancer risk. According to the obtained values, which were all more than 10^{-4}, appropriate solutions should be adopted in this field (Table 8).

Table 8: TR levels of lead (Pb), cadmium (Cd) and arsenic (As) in meat samples of the studied species

As	Cd	Pb	TR
0.00177	0.00152	0.00524	**Sheep**
0.000492	0.513	0.00122	**Beef**
0.0003	0.0000475	0.000155	**Turkey**
0.0002	0.000054	0.000047	**Ostrich**

Discussion

Food contains a wide range of trace elements, some of which are essential and have beneficial nutritional value, and others have toxic effects. Toxic heavy metals, in addition to having negative effects on the growth and productivity of animals, are also transmitted to humans through the food chain and can cause harm to human health. The harm depends on the type of metal, its intake, age, or duration of contact (Rashid et al. ; Yabe et al. 2013). High concentrations of heavy metals in livestock products can be due to pollution of water, food, bedding and the environment (Ayar et al. 2009; Qin et al. 2009). Cadmium, arsenic, and lead are non-ferrous and non-functional metals and are also the most dangerous heavy metals which cause damage to human and animal health. Today, due to the growth of societies and human industries, environmental pollutants can be the main cause of heavy metal poisoning and complications such as cancer (Cunningham and Saigo 1997).

The results of the study showed that there was no significant difference among the four-studied species in the mean content of selenium ($P = 0.571$), nickel ($P = 0.287$), cobalt ($P = 0.351$) and chromium ($P = 0.299$). While the mean content of lead ($P = 0.022$), cadmium ($P = 0.001$), arsenic ($P > 0.001$), zinc ($P > 0.001$) and copper ($P = 0.001$) showed a significant difference in the studied species. These results show that the type of species can affect the accumulation of these metals. Considering the differences in the type of breeding system for cow and sheep compared to turkeys and ostriches, the difference in the type of feeding, slaughter, and transportation conditions, interspecific change between species is justifiable.

The results showed an excessive increase in lead, cadmium and arsenic in beef and sheep. In the case of ostrich meat and turkey, only two cases were more than the permissible amount. The excessive increase of arsenic in the meat of the four studied animals indicates an increase in the level of arsenic in the ecosystem of Kurdistan and its contaminated rural springs. Increased cadmium in mutton and beef indicated contaminated sources of the ecosystem and their unsafe food. Regarding the contamination of cattle and sheep, it seems that most of the livestock in Kurdistan are contaminated due to seasonal and free grazing. The increase in unauthorized levels of lead in mutton, beef and turkey indicated the involvement of environmental pollutants in breeding conditions close to residential areas and human wastewater. Lead, as a toxic compound, can cause kidney failure, changes in hematological parameters, as well as improper changes in the gastrointestinal tract (Baykov et al. 1996; Botkin and Keller 1998). According to the International Agency for Research on Cancer (IARC), lead is a potential carcinogenic compound in humans and its permissible level in water has been reported as 15 ppm, in soil and agricultural products as 0.01 and 0.1 ppm, respectively (Ayeni 2014; Chiroma et al. 2014). Selenium among other metals is receiving special attention. Metabolism, immune responses, and antioxidant properties and playing an important role in the reproductive processes are the brilliant roles of the element. It as an antioxidant can protect against the oxidative damage of other metals (Attia et al. 2010; Hosnedlova et al. 2018; Saleh and Ebeid 2019).

Contrary to the reported increase in selenium in ostrich meat compared to other animal species, the amount of selenium did not show a significant difference between the species studied in this study (NEED references). This issue signified the impact of changes in nutrition, breeding, and storage conditions on the nutritional value of livestock meat. Therefore, its content depending on the type of nutrition, environment and hygienic conditions of breeding can be very important in the nutritional value of meat. There was a significant negative correlation between selenium and cadmium in turkey meat, selenium and arsenic and copper and selenium in ostrich meat. This can be explained by the oxidative stress caused by the accumulation of heavy metals such as lead and the antioxidant role of selenium in this regard. In the present study, the correlation coefficient was negative and showed an inverse relationship between selenium and arsenic, but this amount was not significant. This result was in line with the review study by Ji Sam and Janaysk study. Probably one of the reasons for the decrease in selenium levels in ostrich meat in this study could be attributed to the increase in arsenic levels. In a 2017 study by Janasik et al., They concluded that increased selenium excretion could increase arsenic levels (Janasik et al. 2017). Conclusions in these cases require further studies on species analysis, genetics, and the influence of factors such as nutritional status (Janasik et al. 2017).

Nickel is another essential element for the normal functioning of red blood cells, but increasing its concentration causes dysfunction of the respiratory system, heart and prostate disease (Yabe et al. 2013). The most important way to increase nickel levels is to transfer it from the soil (Hu et al. 2018). In addition to the importance of cobalt in the supply of vitamin B12, cobalt can cause damage to the hormonal, nervous, and cardiovascular systems (Leyssens et al. 2017). In this study, the content of nickel and cobalt in meat of sheep and beef was within the allowable range, but in turkey and ostrich, the amount of nickel was more than the permissible amount for consumption. Considering the performance of nickel poisoning, this can be a warning to the health of adults. Chromium is produced in the ecosystem during industrial farming activities. It improves nutrition and weight gain in animals, increased its concentration can induce oxidative stress and damage to proteins and DNA (Anderson 2003; Duan and Tan 2013; Lien et al. 1999; Vincent 2001). Elevated chromium levels, in addition to being carcinogenic, disrupt insulin secretion and synthesis, and impair blood sugar control, leading to diabetes (Nkansah and Ansah 2014). Copper is an essential element for the activity of enzymes in the body (Jobling 2012) and has the highest concentration in muscles and liver. However, increasing its concentration can cause gastrointestinal dysfunction, including in the stomach and intestines (Elsharawy and Elsharawy ; Ogwok et al. 2014). Therefore, consumption of livestock products with high levels of copper can be a threat to public health (Hu et al. 2018; Jaishankar et al. 2014). Zinc, chromium and copper had higher levels than allowed in the meat of all four species, which indicates a threat to public health. Unfortunately, in the case of TR, which is a risk factor for carcinogenicity, in the case of lead, cadmium, and arsenic, it had an unacceptable amount and a serious warning for the unhealthy protein sources of this area of the province. In this regard, a rapid review of areas used for grazing, analysis of water and animal feed before use in terms of heavy metal concentrations, sanitary control of the slaughter process and transportation and distribution through the country's regulatory institutions are required.

Conclusion

There was a positive correlation between the content of concentrations of some heavy metals including lead, arsenic, and cadmium in meat tissue with animal species. Concentrations of non-essential heavy metals in the meat of the studied animals were excessive and the THQ and TR were unacceptable, indicating their carcinogenicity.

The concentration of non-essential heavy metals in the meat of the studied animals was more than the permissible consumption range and THQ and TR were found to be unacceptable and carcinogenic. Due to the importance of consuming livestock meat in the human diet, identifying, controlling and monitoring the level of safety and health of livestock products in the western part of the country is a serious warning.

Declarations

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Funding Statement

There was no funding support for this article.

Acknowledgments

We would like to sincerely appreciate the Research Vice-Chancellor at the Islamic Azad University, Sanandaj Branch, for his collaboration to facilitate research.

Ethical Approval

This study does not include ethical interventions.

Consent to participate

Not applicable.

Consent for publication

All authors give consent for publication.

Author contributions

Conceptualization, methodology, and software: Mahdieh Raeeszadeh and Abolfazl Akbari. Data curation and writing-original draft preparation: Mahdieh Raeeszadeh, Hamed gravandi, and Abolfazl Akbari. Visulization and investigation: Mahdieh Raeeszadeh and Hamed Gravandi. Supervision and validation: Mahdieh Raeeszadeh.

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Figures

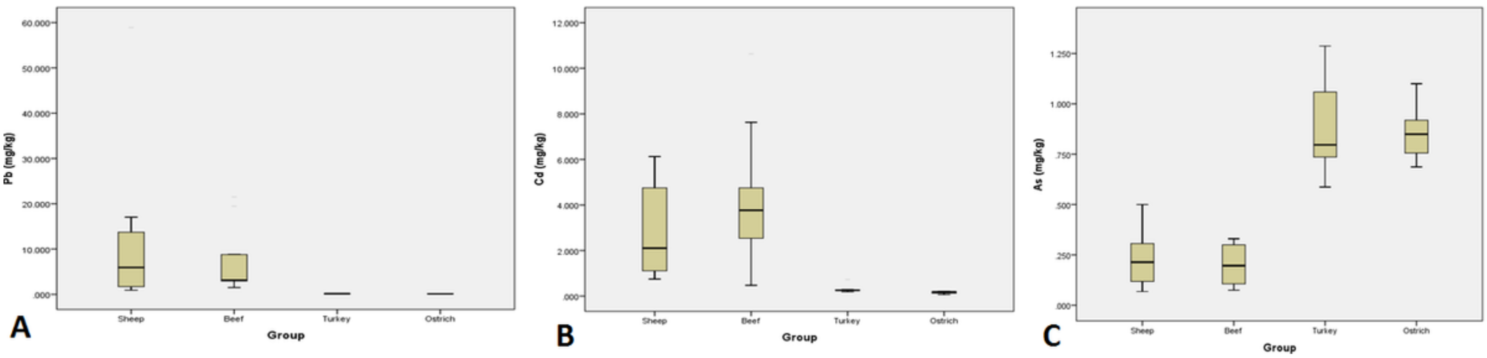

Figure 1

Box plot diagram of accumulated concentrations of lead, cadmium and arsenic by studied species

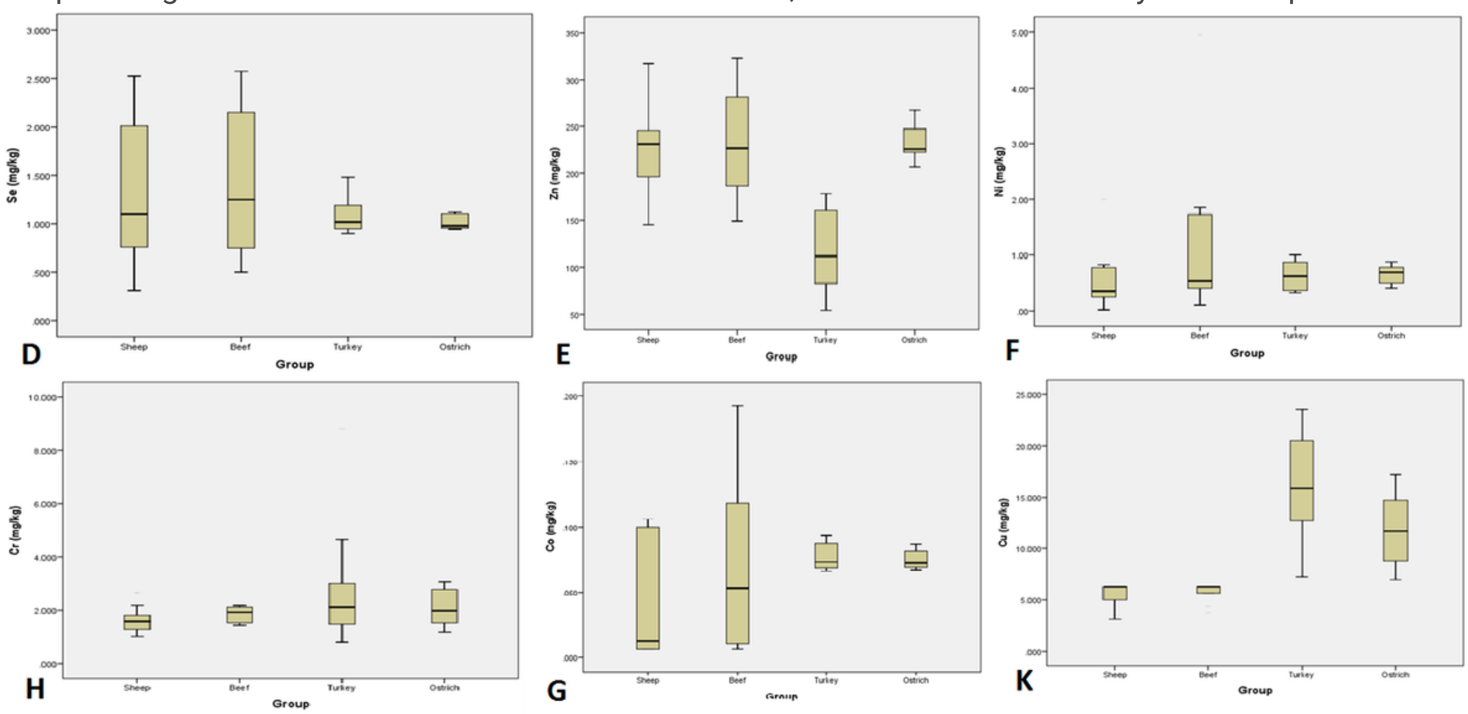

Figure 2

Box plot diagram of the content of selenium, zinc, nickel, cobalt, chromium and copper in the studied species