

Original Article**Heavy Metals and Trace Elements in the Livers and Kidneys of Slaughtered Cattle, Sheep and Goats***Behnaz Bazargani-Gilani^{*1}, Mohamadreza Pajohi-Alamoti¹, Aliasghar Bahari², Abas Ali Sari¹**Received: 20.04.2016**Accepted: 28.05.2016***ABSTRACT**

Background: This study was performed to evaluate the levels of heavy metals in the livers and kidneys of cattle, sheep and goats from a mining region in the west of Iran.

Methods: In this cross-sectional study, 90 samples were collected from the livers and kidneys of cattle (n=30), sheep (n=30) and goats (n=30) from a slaughterhouse in Hamedan City, western Iran. Lead, cadmium, manganese, zinc, copper, and iron concentrations were analyzed using Atomic Absorption Spectroscopy.

Results: Lead concentration exceeded the permissible limit in all samples. Cadmium level was within the permissible limit in most samples, except for the cattle and sheep kidneys. Iron concentration was normal in all samples, except for sheep livers and kidneys. The rest of the elements (manganese, zinc and copper) were within the safe range. There were no sex and age-related significant differences in metal concentrations of cattle samples. The highest effects of age and sex were observed in sheep samples.

Conclusion: The soil of these areas contains high level of lead and cadmium, contaminating the plants, water and animals of these regions; therefore, precise care and health inspection of livestock in these areas are recommended.

Keywords: Environmental Pollution, Heavy Metals, Liver, Kidney, Ruminants.

IJT 2016 (6): 7-13**INTRODUCTION**

Animal tissues such as liver and kidney are important for the human diet, providing essential nutrients for the body [1]. In many Middle East countries, visceral organs (e.g. liver and kidney) are consumed as valuable and palatable food products [2] and in most countries, these products are offered as popular foods in many restaurants [3]. Unfortunately, visceral organs sometimes contain certain toxic compounds such as heavy metals.

Cattle, goat and sheep flocks are usually seen grazing in contaminated lands, where the soil, grass, air and water can be contaminated with heavy metals, entering the livestock tissues [4]. Nowadays, increasing population growth, mining, industrialization, transportation and excessive utilization of natural resources have augmented environmental pollution [5]. Livers and kidneys of livestock are responsible for detoxification of poisonous substances entering the body; thus, they show higher concentrations of these substances

than other organs [1]. Over time, heavy metals can accumulate in the body and become toxic or carcinogenic [6]. Unfortunately, in many countries, local standards for metal levels in food products such as meat and internal organs of animals are missing. Furthermore, contaminated animals with high levels of toxic metals may not show any apparent symptoms of disease and pass health inspection.

The present study aimed at evaluating the levels of heavy metals (lead, cadmium, manganese, zinc, copper, and iron) in the livers and kidneys of cattle, goat and sheep from a slaughterhouse in Hamedan City, western Iran and assessing whether their accumulation is related to sex and age.

MATERIALS AND METHODS

Samples were digested using a modified method [7]. All plastic and glass containers were cleaned by soaking in dilute HNO₃ were rinsed in distilled water and then air dried before use.

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Collection of Samples

Ninety samples was collected from the livers and kidneys of cattle (n=30), sheep (n=30) and goats (n=30) from a slaughterhouse in Hamedan City, western Iran from December 2014 to February 2015. The samples were collected in clean polyethylene bags in an icebox and were transported to the laboratory for analysis. The sex of the animals was recorded at the time of sampling (13 bulls and 17 cows, 14 rams and 16 ewes, and 18 bucks and 12 nannies). Age data were obtained and used to assign animals to separate age groups. Each species was divided into two groups: under 3 yr old (young group) and from 3 yr old (old group). There were 19 young and 11 old cattle, 13 young and 17 old sheep, and 16 young and 14 old goats.

Preparation of Samples

In the laboratory, samples were immediately dissected using a stainless steel dissection instrument. Approximately, 1g wet weight of meat from each sample was dissected and washed with distilled water. The tissue samples were dried in an oven at 105 °C for 24 h. Then, the samples were pulverized into powder by a grinder. Approximately, 0.5 g of each sample was digested by adding 6 ml of nitric acid and 1 ml of hydrogen peroxide (analytical grade) in a digestion tube. Digestion was performed in an electric heater under a chemical hood for 25 min. After

digestion, samples were cooled and the resulting solutions were filtered through Whatman No. 41 filter paper into 10 ml screw-capped polypropylene tubes and the liquid were diluted to the volume of 10 ml with distilled water.

Determination of Heavy Metals

Heavy metals concentrations were assessed using Atomic Absorption Spectroscopy (A.A.S) (Spectr AA 220, Varian). For each heavy metal, there was a specific hollow cathode lamp and the machine was set at a particular wavelength for the metal to be analyzed. The content of heavy metals in the assessed tissues was expressed in mg/kg of fresh mass.

Statistical Analysis

Statistical analysis of the data was conducted using SPSS software (IBM SPSS statistics 21). Tukey and independent-sample *t*-test with a significance value smaller than 0.05 ($P<0.05$) were used to compare the differences among mean values.

RESULTS

Mean and standard errors of heavy metal concentrations in the livers and kidneys of cattle, sheep and goat are shown in Table 1. Tables 2 and 3 present the effect of sex on the heavy metal content in the livers and kidneys. Tables 4 and 5 show age effect.

Table 1. Mean concentrations (mg/kg) and standard errors of heavy metals in the livers and kidneys of cattle, sheep and goats.

Heavy metal	Organ	N	Cattle	Sheep	Goat
Pb	Liver	30 ^a	21.1 ± 3.30 ^{Aa}	17.05 ± 5.17 ^{Aa}	1.69 ± 0.13 ^{Bb}
	Kidney	30	28.10 ± 3.39 ^{Aa}	14.34 ± 4.62 ^{Ab}	2.48 ± 0.17 ^{Ac}
Mn	Liver	30	5.43 ± 0.19 ^{Aa}	6.06 ± 0.39 ^{Aa}	5.37 ± 0.32 ^{Aa}
	Kidney	30	2.89 ± 0.11 ^{Bb}	3.10 ± 0.19 ^{Bab}	3.56 ± 0.18 ^{Ba}
Cd	Liver	30	0.14 ± 0.02 ^{Ba}	0.21 ± 0.4 ^{Ba}	0.01 ± 0.005 ^{Bb}
	Kidney	30	0.93 ± 0.13 ^{Ab}	1.93 ± 0.41 ^{Aa}	0.33 ± 0.06 ^{Ab}
Zn	Liver	30	104.07 ± 5.34 ^{Aa}	105.19 ± 5.11 ^{Aa}	92.73 ± 5.22 ^{Aa}
	Kidney	30	87.94 ± 6.44 ^{Aa}	102.05 ± 5.05 ^{Aa}	92.38 ± 5.25 ^{Aa}
Cu	Liver	30	85.76 ± 8.75 ^{Ab}	126.14 ± 12.93 ^{Aa}	27.93 ± 10.90 ^{Ac}
	Kidney	30	11.91 ± 0.57 ^{Ba}	12.06 ± 0.83 ^{Ba}	7.11 ± 1.49 ^{Ab}
Fe	Liver	30	101.99 ± 8.42 ^{Ab}	199.28 ± 18.94 ^{Aa}	122.66 ± 8.38 ^{Ab}
	Kidney	30	116.15 ± 6.97 ^{Ab}	173.29 ± 12.08 ^{Aa}	137.56 ± 9.01 ^{Ab}

^a Means within the same row (a, b, c) and the same column (A, B) with different letters are significantly different ($P<0.05$).

Table 2. Mean concentrations (mg/kg) and standard errors of heavy metals in the livers of male and female cattle, sheep and goats.

Heavy metal	Sex	N	Cattle (liver)	N	Sheep	N	Goat
Pb	Male	13 ^a	24.49±5.24 ^{Aa}	14	40.44±10.22 ^{Aa}	18	1.68±0.15 ^{Ab}
	Female	17	18.50±4.27 ^{Aa}	16	9.25±4.60 ^{Ba}	12	1.73±0.17 ^{Aa}
Mn	Male	13	5.23±0.29 ^{Aa}	14	4.56±1.14 ^{Aa}	18	5.11±0.33 ^{Aa}
	Female	17	5.59±0.26 ^{Aa}	16	6.56±0.29 ^{Aa}	12	6.84±0.65 ^{Aa}
Cd	Male	13	0.16±0.03 ^{Aa}	14	0.10±0.04 ^{Aab}	18	0.005±0.003 ^{Ab}
	Female	17	0.13±0.02 ^{Aa}	16	0.24±0.06 ^{Aa}	12	0.04±0.02 ^{Aa}
Zn	Male	13	104.75±6.77 ^{Aa}	14	80.86±10.25 ^{Ba}	18	92.57±6.01 ^{Aa}
	Female	17	103.54±8.07 ^{Aa}	16	113.30±4.29 ^{Aa}	12	93.60±9.37 ^{Aa}
Cu	Male	13	79.25±11.85 ^{Aa}	14	45.01±19.83 ^{Bab}	18	23.06±10.33 ^{Ab}
	Female	17	90.74±12.68 ^{Aab}	16	153.23±7.49 ^{Aa}	12	55.57±47.26 ^{Ab}
Fe	Male	13	107.93±14.66 ^{Aa}	14	132.08±26.07 ^{Ba}	18	129.20±8.13 ^{Aa}
	Female	17	97.45±10.10 ^{Ab}	16	221.68±21 ^{Aa}	12	68.72±29.30 ^{Bb}

^a Means within the same row (a, b, c) and the same column (A, B) with different letters are significantly different ($P<0.05$).

Table 3. Mean concentrations (mg/kg) and standard errors of heavy metals in the kidneys of male and female cattle, sheep and goats.

Heavy metal	Sex	N	Cattle (kidney)	N	Sheep	N	Goat
Pb	Male	13 ^a	26.29±4.76 ^{Aa}	14	27.66±11.04 ^{Aa}	18	2.45±0.20 ^{Ab}
	Female	17	29.49±4.86 ^{Aa}	16	9.90±4.62 ^{Aab}	12	2.6±0.4 ^{Ab}
Mn	Male	13	2.94±0.20 ^{Aa}	14	3.00±0.77 ^{Aa}	18	3.54±0.21 ^{Aa}
	Female	17	2.85±0.14 ^{Ab}	16	3.13±0.10 ^{Aab}	12	3.67±0.37 ^{Aa}
Cd	Male	13	1.14±0.27 ^{Aa}	14	0.59±0.18 ^{Aab}	18	0.27±0.05 ^{Bb}
	Female	17	0.77±0.11 ^{Aa}	16	2.38±0.50 ^{Aa}	12	0.68±0.27 ^{Aa}
Zn	Male	13	88.09±7.59 ^{Aa}	14	90.10±12.13 ^{Aa}	18	88.94±5.59 ^{Aa}
	Female	17	87.82±9.97 ^{Aa}	16	106.04±5.26 ^{Aa}	12	111.91±10.29 ^{Aa}
Cu	Male	13	12.29±0.66 ^{Aa}	14	10.01±0.63 ^{Aa}	18	7.06±1.67 ^{Aa}
	Female	17	12.20±0.81 ^{Aab}	16	12.74±1.03 ^{Aa}	12	7.34±3.81 ^{Ab}
Fe	Male	13	116.20±10.75 ^{Aa}	14	117.71±18.12 ^{Ba}	18	134.30±10.25 ^{Aa}
	Female	17	116.12±9.44 ^{Ab}	16	191.82±11.62 ^{Aa}	12	156.04±13.66 ^{Ab}

^a Means within the same row (a, b, c) and the same column (A, B) with different letters are significantly different ($P<0.05$).

Table 4. Mean concentrations (mg/kg) and standard errors of heavy metals in the livers of younger and older cattle, sheep and goats.

Heavy metal	Age	N	Cattle (liver)	N	Sheep	N	Goat
Pb	< 3	19 ^a	19.92±4.05 ^{Ab}	13	40.44±10.22 ^{Aa}	16	1.65±0.15 ^{Ac}
	3	11	23.46±5.95 ^{Aa}	17	9.25±4.60 ^{Ba}	14	1.86±0.24 ^{Aa}
Mn	< 3	19	5.33±0.28 ^{Aa}	13	4.56±1.14 ^{Aa}	16	5.11±0.33 ^{Ba}
	≥ 3	11	5.63±0.20 ^{Aa}	17	6.56±0.29 ^{Aa}	14	6.88±0.55 ^{Aa}
Cd	< 3	19	0.14±0.02 ^{Aa}	13	0.10±0.04 ^{Aab}	16	0.008±0.004 ^{Ab}
	3	11	0.15±0.03 ^{Aa}	17	0.24±0.06 ^{Aa}	14	0.03±0.03 ^{Aa}
Zn	< 3	19	100.06±5.55 ^{Aa}	13	80.86±10.25 ^{Ba}	16	94.81±6.02 ^{Aa}
	≥ 3	11	112.08±11.64 ^{Aa}	17	113.30±4.29 ^{Aa}	14	80.95±1.97 ^{Aa}
Cu	< 3	19	78.09±9.49 ^{Aa}	13	45.01±19.83 ^{Bab}	16	26.20±11.90 ^{Ab}
	> 3	11	95.93±17.09 ^{Aab}	17	153.18±7.51 ^{Aa}	14	37.74±32.29 ^{Ab}
Fe	< 3	19	103.04±10.92 ^{Aa}	13	132.08±26.07 ^{Ba}	16	127.92±8.38 ^{Aa}
	> 3	11	99.89±13.63 ^{Ab}	17	221.68±21.00 ^{Aa}	14	92.84±27.34 ^{Ab}

^a Means within the same row (a, b, c) and the same column (A, B) with different letters are significantly different ($P<0.05$).

Table 5. Mean concentrations (mg/kg) and standard errors of heavy metals in the kidneys of younger and older cattle, sheep and goats.

Heavy metal	Age	N	Cattle (Kidney)	N	Sheep	N	Goat
Pb	< 3	19 ^a	26.14±3.89 ^{Aa}	13	27.66±11.04 ^{Aa}	16	2.49±0.20 ^{Ab}
	3	11	32.04±6.69 ^{Aa}	17	9.90±4.62 ^{Ab}	14	2.40±0.41 ^{Ab}
Mn	< 3	19	2.87±0.16 ^{Aa}	13	3.00±0.77 ^{Aa}	16	3.64±0.21 ^{Aa}
	≥ 3	11	2.91±0.16 ^{Aa}	17	3.13±0.10 ^{Aa}	14	3.12±0.08 ^{Aa}
Cd	< 3	19	1.01±0.19 ^{Aa}	13	0.59±0.18 ^{Ab}	16	0.28±0.05 ^{Ab}
	3	11	0.77±0.11 ^{Aa}	17	2.38±0.50 ^{Aa}	14	0.62±0.29 ^{Aa}
Zn	< 3	19	87.25±7.56 ^{Aa}	13	90.10±12.13 ^{Aa}	16	94.00±4.66 ^{Aa}
	≥ 3	11	89.32±12.61 ^{Aa}	17	106.04±5.26 ^{Aa}	14	83.21±26.74 ^{Aa}
Cu	< 3	19	12.17±0.70 ^{Aa}	13	10.01±0.63 ^{Ab}	16	6.75±1.62 ^{Ab}
	3	11	12.36±0.79 ^{Aa}	17	12.74±1.03 ^{Aa}	14	9.1±4.56 ^{Aa}
Fe	< 3	19	117.21±8.85 ^{Aa}	13	117.71±18.12 ^{Ba}	16	138.74±9.89 ^{Aa}
	≥ 3	11	114.04±11.76 ^{Ab}	17	191.82±11.62 ^{Aa}	14	130.92±26.22 ^{Ab}

^aMeans within the same row (a, b, c) and the same column (A, B) with different letters are significantly different ($P < 0.05$).

DISCUSSION

Lead

Table 1 presents the results of lead concentration in the livers and kidneys of all assessed species. The lead was present in all samples. The concentration of lead was higher in the cattle than the sheep and goats. Lead concentration in all specimens was higher than the permissible limit (>1 ppm) [8]. Other researchers reported such contamination in the livers and kidney of cattle, beef, mutton, goat, sheep, buffalo and chicken in other regions as well [9,3]. There was no significant difference in the lead concentration between male and female species, except for the liver of male sheep that contained significantly ($P < 0.05$) higher lead levels than those of female sheep (Tables 2 and 3). Age factor in lead level followed this pattern as well and only the liver of young sheep showed a significant ($P < 0.05$) difference with old ones. Similar results were reported as for deer [10]. However, some authors found age and sex-related differences in animals [9].

Cadmium

Cadmium was observed in all samples (Table 1). The highest concentration of cadmium was in the sheep. The kidney of sheep (1.93 mg/ml) and the liver of goat (0.01 mg/ml) had the highest and lowest cadmium concentrations, respectively. Except for the cattle (0.93 mg/ml) and sheep kidney (1.93 mg/ml), cadmium concentration in all species was lower than the permissible limit (< 0.5 ppm) [11]. Cadmium levels in animal kidneys were significantly higher than their livers ($P < 0.05$) (Table 1). This could be

due to the excretory function of kidneys. High levels of lead and cadmium in these samples can be correlated to the existence of lead and cadmium mines in this area [12]. This region includes parts of Malayer, Tuyserkan and Asadabad cities, located around Hamedan City (Fig.1). Therefore, considerable amounts of lead and cadmium exist in the soil of these areas that could enter into farm animal feeds and tissues. According to Table 2 and 3, there was no significant difference in cadmium concentration between male and female species, except for the kidney of female goat that contained significantly ($P < 0.05$) higher levels of cadmium than those of male goats. There was also no significant difference in cadmium concentration between old and young animals of all species (Tables 4 and 5). Khan et al. reported no sex and age-related differences in cadmium concentrations in livers and kidneys of the studied animals [10], but several studies have reported age and sex differences in cadmium concentration of cattle, sheep and goats [9,13].



Figure 1. Areas with high numbers of heavy metal mining operations.

Manganese

The main organ containing manganese accumulation was the liver with statistically significant ($P < 0.05$) higher levels than the kidneys (Table 1). All tested animals had liver manganese concentrations below accepted toxic levels (23 mg/kg wet weight) and above those described as deficient (< 1 mg/kg wet weight). Although manganese can exhibit toxic effects in higher concentrations, it is often considered one of the least toxic trace elements in mammals. In fact, homeostatic mechanisms keep tissue manganese levels within a limited range [14]. The mean concentrations of manganese in the livers and kidneys of males and females are shown in Table 2 and 3, respectively. Manganese concentrations in the liver were higher in females than males; whereas, the results were similar in both groups for the kidney. This difference was probably the result of the liver being the main tissue for manganese accumulation [14]. There is little information on the influence of sex on manganese accumulation. Similar results in goats is reported [14]. Table 4 and 5 show mean concentrations of manganese in the livers and kidneys of younger and older animals. According to the results, manganese level in livers and kidneys of older cases was slightly, but insignificantly higher than the younger ones, except for the goat livers. In agreement with our findings, several authors recorded no age differences in manganese concentrations in deer, domestic and laboratory animals [10,15].

Zinc

Table 1 presents the results of zinc concentration in the livers and kidneys of our cases. The highest zinc level (105.19 mg/kg) was found in the liver of the sheep and the lowest level (87.94 mg/kg) was in the kidneys of the cattle. Zinc predominantly accumulates in the liver and then the kidney. Previous researchers described a similar pattern of zinc accumulation in their studies [16, 17]. All values in our studied samples were below the permissible limit (< 150 ppm), set by Australian and New Zealand Food Authority (ANZFA) [8]. Low concentration of zinc may be attributed to zinc deficient farm soils. Perhaps, this is one of the reasons for low tissue content of zinc. Zinc is controlled by precise homeostatic mechanisms for gastrointestinal absorption and excretion; consequently, it has little variation in

concentration. No significant difference was observed in zinc levels of liver and kidney between studied species. According to Tables 2 and 3, there was no significant effect of sex on zinc concentrations in the livers and kidneys of all species, except for the sheep liver. The zinc level of liver in female sheep was significantly ($P < 0.05$) higher than that of male livers. A similar pattern was observed for age factor (Table 4 and 5). Only the zinc level of liver in older sheep was significantly ($P < 0.05$) higher than the younger ones. These results were in agreement with the findings regarding sex and age factors on zinc levels in livers and kidneys of animals in previous studies [14,18].

Copper

Copper concentrations in the tissues of different species are shown in Table 1. The highest copper level (126.14 mg/kg) was found in the sheep liver, while its lowest level (7.11 mg/kg) was reported for the goat kidney. Copper concentrations in all samples were lower than the permissible limit of 200 ppm [8]. Copper level in liver samples was significantly higher than kidney samples in all species ($P < 0.05$). Liver was the main organ containing copper, followed by the kidney, muscle, and blood, concurs with previous studies [14,19]. According to metal richness and total rank score, organs had the following order: liver $>$ kidneys $>$ heart $>$ lungs $>$ muscles. The livers and kidneys are target tissues for monitoring metal contamination in animals because both organs are responsible for removing toxic metals from the body [3]. Furthermore, livers and kidneys of the sheep contained the highest ($P < 0.05$) copper level among the samples, followed by cattle and goats. Ruminants, particularly the sheep, have higher potentials for copper accumulation in their liver than other species and are more susceptible to copper toxicity [14]. According to Tables 2 and 3, sex did not affect copper accumulation in the livers and kidneys of cattle and goats [10,14]; whereas the liver of female sheep contained significantly ($P < 0.05$) higher copper levels than the male sheep. Age effect was similar to sex. Copper level in the liver of older sheep was significantly ($P < 0.05$) higher than the younger ones (Tables 4 and 5). Other studies reported no age dependence of copper concentration in deer, domestic and laboratory animals [15].

Iron

Iron concentrations are summarized in Table 1. The highest concentration (199.28 mg/kg) was found in the sheep livers while the lowest (101.99 mg/kg) concentration was observed in cattle's liver. The permissible limit of iron in food is generally 30-150 mg/kg [8], but in sheep livers and kidneys, it exceeded this range. Unlike sheep, iron accumulated preferentially in the kidneys and then the livers of the cattle and goats. A similar pattern of accumulation was reported by Falandysz [2]. In contrast, Erdogan et al. found higher iron accumulation in the liver than in the kidney [20]. Iron levels in the livers and kidneys of males and females were different for all species (Tables 2 and 3). No significant difference was seen between the livers and kidneys of male and female cattle, whereas the livers and kidneys of female sheep contained significantly ($P < 0.05$) higher iron levels than the male sheep. Iron concentration of liver in male goats was significantly ($P < 0.05$) higher than female goats, but there was no significant difference between male and female goats kidneys.

There is not enough data regarding the effect of sex on iron accumulation in animals. Khan et al. reported significantly higher iron concentrations in the livers and kidneys of male goats [21], whereas contrasting results have been reported for the cattle [14]. Tables 4 and 5 present the effects of age on iron levels in different species. Cattle and goats did not show any significant differences in iron concentrations of livers and kidneys of younger and older animals, whereas the livers and kidneys of older sheep contained significantly ($P < 0.05$) higher iron level than the younger sheep.

CONCLUSION

The soil of these areas contains high level of lead and cadmium, contaminating the plants, water and animals of these regions; therefore, precise care and health inspection of livestock in these areas are recommended. The effect of both sex and age on the exposure to heavy metals has been well documented and it has been proven how they affect levels of metals. Detailed investigations are required to identify the reasons of these findings.

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