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Incidence and public health risk assessment of toxic metal residues (cadmium and lead) in Egyptian cattle and sheep meats

[1,2]Darwish, W. S., [1]Hussein, M. A., [3]El-Desoky, K. I., [2]Ikenaka, Y., [2]Nakayama, S. [2]Mizukawa, H. and [2*]Ishizuka, M.

[1]*Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt*
[2]*Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.*
[3]*Veterinary Animal Hospital, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt*

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Abstract

Environmental pollution by heavy metals is a serious problem worldwide. This study aimed to investigate comparisons between concentrations of toxic metals (cadmium and lead) in the liver, kidneys, and muscle of Egyptian cattle and sheep. The effect of animal age on levels of toxic metal residues as well as results of a public health risk assessment is also reported. The results show that both cadmium and lead levels exceeded Food and Agriculture Organization and World Health Organization permissible limits in the liver and kidneys. These parts had higher concentrations of toxic metals compared with muscle in the two animal species examined. Age had a significant influence on toxic metal accumulation in both species. The hazard index indicates that consuming the livers and kidneys of these animals might pose a health risk.

Introduction

Environmental pollution by heavy metals is a serious problem in most countries worldwide. Heavy metals can be found in food, water, soil, air, plant, and animal tissues, particularly in the liver and kidneys (Abou-Arab, 2001). Monitoring programs are being carried out in many countries with the purpose of avoiding the distribution of foodstuffs that could pose a risk to human health if consumed (Milićević *et al.*, 2009). As Egypt is one of the most populous countries in Africa, with an estimated population of 85 million, environmental pollution is considered one of the most dangerous hazards affecting the country. Pollution by heavy metals has increased because of increases in population, the number of agricultural projects, as well as industrial and other activities along the Nile Delta (Darwish *et al.*, 2010).

Food consumption is considered the major source of human exposure to heavy metals. Biomarkers must be established to monitor the impact of these pollutants on public health. Cattle and sheep are considered accurate biomarkers for different environmental pollutants, particularly heavy metals, as they have the ability to accumulate toxic metal residues. Moreover, these animals are domesticated and live within the same environments as humans do (Darwish *et al.*, 2010).

Heavy metals are significantly toxic, due to their cumulative nature in the different body organs leading to adverse effects (Jarup, 2003; Sathawara *et al.*, 2004). Metals tend to bioaccumulate in the environment and biomagnify in food chains (Caggiano *et al.*, 2004), their levels might reach toxic limits even when found in low concentrations in environmental samples. Since this should be limited to an unavoidable minimum, much attention is paid to the occurrence of these elements in food (Milićević *et al.*, 2009). Heavy-metal levels in animal tissues are tissue-specific. In many Middle Eastern countries, particularly Egypt, offal such as liver, lungs, kidneys, rumen, intestine, tongue, spleen, and heart are sold and consumed as a valuable food source: however, residual levels of heavy metals in offal are rarely measured in Egypt. Therefore, evaluating heavy-metal levels in cattle and sheep offal as well as muscle is important for safety and health purposes (Darwish *et al.*, 2010; Yabe *et al.*, 2010). The Sharkia Province is located north to Cairo, the capital of Egypt, and the Egyptian population in Sharkia depends mainly on agricultural and small industrial activities; therefore, many areas of the province have been contaminated

*Corresponding author.
Email: *ishizum@vetmed.hokudai.ac.jp*
Tel: +81-11-706-6949; Fax: +81-11-706-5105

with wastewater and solid wastes. Cattle and sheep herds in Egypt have an open access for grazing in and around contaminated areas, with meat and dairy products from these animals reaching local markets without any monitoring of heavy-metal levels. Therefore, the present study aimed to evaluate levels of the heavy metals cadmium and lead in cattle and sheep tissues marketed at Sharkia Province markets. Additionally, a human health risk assessment was conducted on the consumption of meat from these animals.

Increasing of the animal age is usually associated with decreases in the water and protein content of meat as well as increases in fat and ash content. This leads to an increase in levels of contamination by heavy metals, especially by lead and cadmium (Pitot and Dragan, 1995), with possible implications for food safety. However, there is limited information concerning the effect of animal age and heavy-metal levels, as well as its implications for safety and its availability for human consumption of contaminated meat.

The current study aimed to compare levels of toxic metal residues (cadmium and lead) in the liver, kidneys, and muscles of Egyptian cattle and sheep. Special reference was given to a public health risk assessment. Additionally, the study examined the effect of animal age on accumulation patterns of cadmium and lead in various cattle and sheep tissues.

Materials and Methods

Sampling

A total of 120 random samples of liver, kidneys, and thigh muscles (10 of each) were collected from carcasses of young and aged male sheep (Ovis aries) (Baladi breed) and male cattle (Bos taurus) (native breed). Samples (20 g of each) were examined clinically and inspected at Zagazig Abattoir, Zagazig, Sharkia. Samples were collected in the period of September 2010 to May 2011. Cattle samples classified as young were from animals 2.00 ± 0.46 years of age; aged cattle were 5.00 ± 0.56 years of age. Thirty samples in were taken from each age group (10 each of liver, kidneys, and muscles). Sheep samples classified as young were from animals 9.00 ± 0.54 months years of age; aged sheep were 2.3 ± 0.56 years of age. Thirty samples for were also taken from each age group (10 each of liver, kidneys, and muscles). Young and aged classifications were dependent on the appearance of permanent teeth.

Sample preparation and extraction

Sampled tissues for analysis were stored in plastic falcon tubes and placed in a cooler box. Samples in the box were stored at −20°C during transfer to the laboratory up until sample preparation and analyses. Heavy-metal levels in examined tissues were measured according to the method described by Finerty *et al*. (1990). In summary, 1 g of each sample was mixed with 10 mL 3:2 HNO_3 (65%v/v): HClO4 (70%v/v). The mixture was allowed to digest overnight in the cold and later heated for 3 h in a water bath at 70ºC with swirling at 30 min intervals to ensure complete digestion. After cooling, the digest was transferred into 20 mL standard flasks, rinsing with de-ionized water and made up to the mark. Prepared sample solutions were kept in acid-leached polyethylene bottles at room temperature until metal analyses.

All reagents used were analytical grade and standard solutions of lead and cadmium were purchased from Merck, Darmstadat, Germany. Metals concentrations were measured using an atomic absorption spectrophotometer (PerkinElmer 2380), using hollow cathode lamps, equipped with air-acetylene flame, the level of lead (Pb) was measured at 217 nm, while that of cadmium (Cd) was measured at 228.8 nm.

The accuracy of the analysis was checked by measuring IAEA-142/TM from IAEA's certified reference materials, Vienna, Austria (muscle homogenate). Mean recoveries ranged from 97 to 104%. Recovered concentrations of the certified samples were within 5% of the certified values. The detection limits of Pb and Cd were 0.05 and 0.02 µg/g respectively. Samples were analyzed in triplicates.

Estimated daily intake (EDI)

The EDI was calculated based on integration of data from analysis of heavy metals, meat consumption rates, and body weight of Egyptian adults.

EDI (µg/kg/day) for both lead and cadmium was obtained using the following equation described by the Human Health Evaluation Manual (US Environmental Protection Agency, EPA) (2010):

$$EDI = \frac{C_m \times F_{IR}}{BW}$$

Where C_m is the concentration of the metal in the sample (mg/kg wet weight); FIR is the food (meat) ingestion rate in Sharkia, Egypt, which was estimated at 85.7 g/day (FAO 2003); BW is the body weight of Egyptian adults, which was estimated at 70 kg.

Health risk assessment

The US EPA (1989) quantitatively assesses human health risks in terms of non-cancer and cancer risks. This study aimed to quantify the non-cancer risk imposed on Sharkia, Egypt, by consumption of metal-contaminated meats. The risk assessment followed the guidelines recommended by the US EPA (2010). For non-carcinogenic effects, the EDI was compared with the recommended reference doses (RfD) (0.001 mg/kg/day for Cd and 0.004 mg/kg/day for Pb) (US EPA, 2010), as stated in the following equation:

$$\text{Hazard Ratio (HR)} = \frac{\text{EDI}}{\text{RfD}} \times 10^{-3}$$

The hazard ratios (HRs) can be summed to generate a hazard index (HI) to estimate the risk of mixed contaminants. HI was generated by using the following equation:

$$\text{HI} = \sum \text{HR}i$$

where i represents each metal
A HR and/or HI of >1 indicates that there is potential risk to human health, whereas a result of ≤1 indicates no risk of adverse health effects.

Statistical analysis

Statistical significance was evaluated using Tukey–Kramer honestly significant difference tests, with $p < 0.05$ considered as significant. Correlation analyses were performed using JMP program (SAS Institute, Cary, NC, USA).

Results and Discussion

Heavy-metal content of cattle and sheep meats

The present study measured residual concentrations (ppm/wet weight) of cadmium and lead in the liver, kidney, and muscle tissues of young and aged cattle and sheep. The mean cadmium concentrations for young and aged cattle were 0.173 ± 0.012 and 0.298 ± 0.067, respectively in the liver samples, and 0.226 ± 0.027 and 0.627 ± 0.176, respectively in the kidney samples. Mean cadmium concentrations for muscle samples were 0.006 ± 0.001 in young cattle, and 0.009 ± 0.001 in aged cattle (Table 1). The mean cadmium concentrations for young and aged sheep were 0.115 ± 0.012 and 0.203 ± 0.014, respectively in the liver samples, and 0.137 ± 0.014 and 0.337 ± 0.0106, respectively in the kidney samples (Table 1). Residual lead concentrations of 0.373± 0.051 and 0.603 ± 0.096 in young and aged cattle livers were respectively recorded, and concentrations of 0.234 ± 0.030 and 0.485 ± 0.153 for young and aged sheep livers, were respectively recorded (Table 2). Residual lead concentrations in kidney samples of young and aged cattle were 0.322 ± 0.056 and 0.609 ± 0.868 ppm of lead respectively, whereas concentrations in kidney samples of young and aged sheep were 0.170 ± 0.032 and 0.502 ± 0.076, respectively (Table 2).

From these results, clear interspecies differences in the accumulation of heavy-metal residues between cattle and sheep tissues were observed. Cattle, especially at a younger age, had higher levels of both cadmium and lead than did sheep. This may be due the longer life span and larger size of cattle compared with those of sheep. Additionally, cattle are the major source of animal food in Egypt; therefore, the number of slaughtered cattle is much higher than that of slaughtered sheep. Slaughtered cattle are usually transported from abattoirs in open trucks, which pose a high risk of contamination of the meats by vehicular smoke, which contains high levels of lead and cadmium.

Examined samples exceeding the MPLs of heavy metals clearly showed either a tissue-, metal-, or age-specific trend. Interestingly, all examined liver and kidney samples had higher levels of lead than the FAO/WHO, MPLs, especially in the aged group. The present study recorded levels of both cadmium and lead higher than those in a previous report in Egypt (Abou-Arab, 2001), as well as in reports published in Brazil, Slovenia, Spain, and Iran (Aranha *et al.*, 1994; Doganoc, 1996; Lopez-Alonso, 2002; Rahimi and Rokni, 2008). The high levels of cadmium and lead in the current study compared with those reported by Abou-Arab, (2001), may be attributed to the extensive use of these two metals in the Egyptian industry, such as in nickel–cadmium batteries, anticorrosive coating of metals, and pigments and stabilizers for plastic. Significant quantities of cadmium and lead are also released from human activities (Baldini *et al.*, 2000; Rahimi and Rokni, 2008), with 90% of atmospheric cadmium and lead emissions coming from anthropogenic sources. These emissions, together with the release of cadmium and lead into aquatic and terrestrial environments, may lead to severe local pollution (Jill *et al.*, 2001). Moreover, airborne cadmium and lead may spread widely in the environment by long-range atmospheric transport (Pitot and Dragan, 1995; Yabe *et al.*, 2010).

The results show that both kidney and liver tissues had greater levels of cadmium and lead compared with those in muscle, and thus correspond with previous reports (Abou-Arab, 2001; Rahimi and Rokni, 2008). These results may be plausible, as both organs are responsible for the detoxification and excretion of xenobiotics (Pitot and Dragan, 1995).

Table 1. Cadmium content (ppm/wet wt.) in various tissues of cattle and sheep slaughtered at Zagazig City Abattoir, Egypt

		Cattle			Sheep		
		Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE
Liver	Young	0.122	0.250	$0.173 \pm 0.012^{\#, A}$	0.055	0.190	0.115 ± 0.012^{A}
	Aged	0.110	0.686	0.298 ± 0.067^{b}	0.159	0.257	$0.203 \pm 0.014^{*, b}$
Kidney	Young	0.120	0.348	$0.226 \pm 0.027^{\#, A}$	0.075	0.213	0.137 ± 0.014^{A}
	Aged	0.004	1.757	$0.627 \pm 0.176^{*, a}$	0.058	0.575	$0.337 \pm 0.106^{*, a}$
Muscle	Young	0.001	0.009	0.006 ± 0.001^{B}	0.001	0.009	0.003 ± 0.001^{B}
	Aged	0.008	0.014	$0.009 \pm 0.001^{*, ab}$	0.001	0.016	0.006 ± 0.002^{c}

$^{\#}$ Superscript implies significant difference between the mean values for cattle and sheep in the same row.
* Superscript implies significant difference between the mean values of the same tissues of young and aged animals of the same species.
$^{A, B}$ Superscript implies significant difference between various tissues (liver, kidney, and muscle) of young animals in the same column.
$^{a, b, c}$ Superscript implies significant difference between the various tissues (liver, kidney, and muscle) of aged animals in the same column.

Table 2. Lead content (ppm/wet wt.) in various tissues of cattle and sheep slaughtered at Zagazig City Abattoir, Egypt

		Cattle			Sheep		
		Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE
Liver	Young	0.129	0.550	$0.373 \pm 0.051^{\#, A}$	0.012	0.390	0.234 ± 0.030^{A}
	Aged	0.200	1.065	$0.603 \pm 0.096^{*, a}$	0.155	0.760	$0.485 \pm 0.153^{*, ab}$
Kidney	Young	0.116	0.570	$0.322 \pm 0.056^{\#, AB}$	0.075	0.235	0.170 ± 0.032^{A}
	Aged	0.280	1.030	$0.609 \pm 0.868^{*, a}$	0.240	0.864	$0.502 \pm 0.076^{*, a}$
Muscle	Young	0.075	0.400	$0.168 \pm 0.033^{\#, B}$	0.050	0.130	0.078 ± 0.023^{B}
	Aged	0.110	0.686	0.298 ± 0.067^{b}	0.020	0.484	$0.258 \pm 0.081^{*, b}$

$^{\#}$ Superscript implies significant difference between the mean values for cattle and sheep in the same row.
* Superscript implies significant difference between the mean values of the same tissues for young and aged animals of the same species.
$^{A, B}$ Superscript implies significant difference between the various tissues (liver, kidney, and muscle) of young animals in the same column.
$^{a, b}$ Superscript implies significant difference between the various tissues (liver, kidney, and muscle) of aged animals in the same column.

Effect of age on heavy-metal accumulation in cattle and sheep meats

The present study observed a clear positive correlation between age and accumulation of cadmium and lead in cattle and sheep meats (Figures 1 and 2). Cadmium content in kidneys was 2.77 times higher in aged cattle than in young cattle, while the content in muscles was only 1.5 times higher in aged cattle than in young cattle. Cadmium levels in the kidneys and liver of aged sheep were 2.45 and 1.76 times higher than those in young sheep. Lead levels in the liver and kidneys of aged cattle were 1.61 and 1.89 times higher than those in young cattle. However, lead levels in kidneys, muscle, and liver of aged sheep were 2.9, 3.3, and 2.07 times higher than those in young sheep. This age-dependent increase in the heavy-metal content of examined livers, kidneys, and muscle is consistent with results of previous research (Rahimi and Rokni, 2008).

The correlation coefficient between cadmium and lead showed negative or no correlations between the two metals in the various cattle tissues examined.

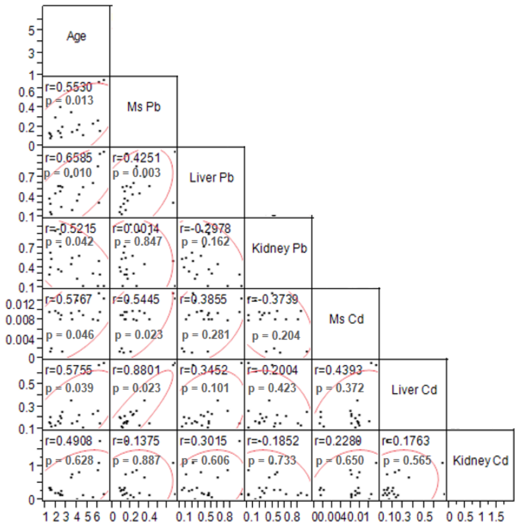

Figure 1. Correlation analysis between age and cadmium and lead accumulation in the various tissues of cattle slaughtered at Zagazig Abattoir, Egypt
Multivariate correlation analysis between age and accumulated lead and cadmium in the various tissues (muscle, liver, and kidney) of slaughtered cattle (n = 20). Analysis was done using JMP program (SAS Institute, Cary, NC, USA). *r* refers to the Pearson correlation coefficient, and p refers to the P value; $p < 0.05$ was considered significant

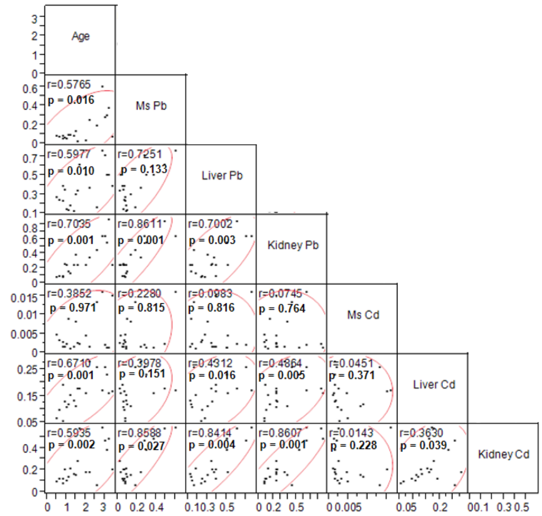

Figure 2. Correlation analysis between age and lead and cadmium accumulation in the various tissues of sheep slaughtered at Zagazig Abattoir, Egypt
Multivariate correlation analysis between age and accumulated lead and cadmium in the various tissues (muscle, liver, and kidney) of slaughtered cattle (n = 20). Analysis was done using JMP program (SAS Institute, Cary, NC, USA). *r* refers to the Pearson correlation coefficient, and *p* refers to the P value; $p < 0.05$ was considered significant

In contrast, strong positive correlations between the two elements were observed in the kidneys and livers of sheep. This could be attributed to the difference in feeding behaviors between sheep and cattle. Sheep are usually classified as active grazers in rainy or dry seasons, whereas cattle need to receive food in a farm. This difference allows sheep to ingest both the upper parts and roots of the plant, causing them to have the same patterns of accumulation for both lead and cadmium.

Daily intake and human risk assessment

The persistent occurrence and accumulation of heavy metals, particularly lead and cadmium, and the potential exposure to humans, from numerous sources such as food, water, soil and air, make them ranked by Agency of Toxic Substances and Disease Registry (ATSDR) as most hazardous and toxic substances in the environment (ATSDR, 2011). Recently, lead is encountered in many poisoning cases especially in children in many locations such as in Bagega community, Zamfara, Nigeria (Ajumobi *et al.*, 2014); in Kabwe, Zambia (Yabe *et al.*, 2014) and in Changchun, Jilin Province, China (Xu *et al.*, 2014). In the current study, all samples from both young and aged cattle had lead concentrations exceeding the Food and Agriculture Organization (FAO)/ World Health Organization (WHO, 2004) maximum permissible limits (MPLs) (0.1 and 0.2 ppm for Cd and Pb respectively), as indicated in Table 3. All sheep liver samples had levels of lead higher than the FAO/WHO MPL (Table 3). Muscle samples from young and aged cattle and sheep did not exceed the FAO/WHO MPL for cadmium (Table 3).

The mean value for the EDI of cadmium in cattle samples ranged from 0.007 to 0.767 µg/kg/day (Table 4). The HR for mean cadmium content in examined tissues ranged from 0.007 to 0.767: however, there were aged kidney samples having the highest concentrations of cadmium with HR of 2.151, whereas the EDI of lead ranged from 0.205 to 0.745 (Table 4). The HR for mean lead concentrations ranged from 0.051 to 0.186 (Table 4). The highest HI value for ingestion of cattle meats contaminated with cadmium and lead was 2.466 in aged kidney samples, followed by 1.165 in aged livers (Table 4). The HR values recorded in our study were lower than Min *et al.* (2012), who recorded a hazard ratio for lead as 1.24 and for cadmium as 1.27 and suggested that high blood lead and cadmium levels in human body may be associated with balance and vestibular dysfunction in a general sample of U. S. adults. Unlikely, lower values were reported by Yu et al. (2014), who recorded 0.58 and 0.28 as HI for children and adults

Table 3. Percentages of samples exceeding FAO/WHO maximum permissible limits (MPLs) for lead and cadmium (ppm) in meats

		Cattle		Sheep	
		Cd	Pb	Cd	Pb
Liver	Young	30%	100%	0%	100%
	Aged	60%	100%	40%	100%
Kidney	Young	50%	100%	10%	60%
	Aged	60%	100%	50%	100%
Muscle	Young	0%	70%	0%	40%
	Aged	0%	100%	0%	50%

Table 4. Estimated daily intakes (EDIs) (µg/kg/day), hazard ratios (HRs), and hazard indexes (HIs) of the mean and highest concentrations of cadmium and lead from consumption of cattle meats in Sharkia, Egypt

		EDI				HR				HI	
		Cd		Pb		Cd		Pb		Cd + Pb	
		Mean	High	Mean	High	Mean	High	Mean	High	Mean	High
Liver	Young	0.211	0.306	0.456	0.673	0.211	0.306	0.114	0.168	0.325	0.474
	Aged	0.364	0.839	0.738	1.303	0.364	0.839	0.184	0.325	0.549	1.165
Kidney	Young	0.276	0.426	0.394	0.697	0.276	0.426	0.098	0.174	0.375	0.600
	Aged	0.767	2.151	0.745	1.261	0.767	2.151	0.186	0.315	0.954	2.466
Muscle	Young	0.007	0.011	0.205	0.489	0.007	0.011	0.051	0.122	0.058	0.133
	Aged	0.011	0.017	0.364	0.839	0.011	0.017	0.091	0.209	0.102	0.227

exposed to the toxic metals (arsenic, cadmium, copper, chromium and lead) in the urban street dust of Tianjin, China.

EDI values of cadmium in sheep samples ranged from 0.003 to 0.412 µg/kg/day, whereas EDI values for lead ranged from 0.095 to 0.614 (Table 5). HR and HI values did not exceed 1 for both Cd and Pb in all examined liver samples (Table 5). A tolerable weekly intake (TWI) of 7 µg/kg bw of Cd was set by WHO (WHO 1989), and was reaffirmed in 2003 (WHO, 2003). Our results showed that the estimated mean and high-level exposures to cadmium of all examined samples (equivalent to 1 µg/kg bw/day) were within or slightly higher than the TWI determined by WHO. In 2009, the European Food Safety Authority (EFSA, 2009) published its scientific perspective on cadmium in food, and established a revised TWI for cadmium of 2.5 µg/kg bw (equivalent to 0.36 µg/kg bw per day). The mean dietary exposure across European countries was estimated to be 2.3 µg/kg bw per week, with high-level exposure estimated to be 3.0 µg/kg bw per week. Although adverse effects on kidney function are unlikely to occur at exposures twofold greater than the TWI, the EFSA concluded that exposure to cadmium at the population level should be reduced. Consideration of the recent TWI values for Cd clearly indicates that the consumption of offal may pose a public health risk.

Mean and high-level daily intakes of lead for both cattle and sheep samples were within the tolerable levels established by WHO (2003) on the daily intake (3.6 µg/kg bw per day). The HR and HI for mean levels of Cd and Pb were <1; however, the consumption of high levels of both toxic metals may pose a public health hazard.

Conclusions

Liver and kidneys had higher concentrations of toxic metals compared with muscle in both cattle and

Table 5. Estimated daily intakes (EDIs) (μg/kg/day), hazard ratios (HRs), and hazard indexes (HIs) of the mean and highest concentrations of cadmium and lead from consumption of sheep meats in Sharkia, Egypt

		EDI				HR				HI	
		Cd		Pb		Cd		Pb		Cd + Pb	
		Mean	High	Mean	High	Mean	High	Mean	High	Mean	High
Liver	Young	0.140	0.232	0.286	0.477	0.140	0.232	0.072	0.119	0.212	0.351
	Aged	0.248	0.314	0.593	0.930	0.248	0.314	0.148	0.232	0.397	0.547
Kidney	Young	0.167	0.260	0.208	0.287	0.167	0.260	0.052	0.071	0.219	0.332
	Aged	0.412	0.703	0.614	1.057	0.412	0.703	0.153	0.264	0.566	0.968
Muscle	Young	0.003	0.011	0.095	0.159	0.003	0.011	0.024	0.039	0.027	0.050
	Aged	0.007	0.019	0.315	0.592	0.007	0.019	0.079	0.148	0.086	0.167

sheep. Cattle, especially at a younger age, had higher concentrations of both cadmium and lead compared with those of sheep. Age had a significant influence on accumulation of toxic metals. On the basis of their heavy-metal content, we therefore highly recommend consumption of meat from younger animals. A positive correlation was found between accumulation patterns of cadmium and lead, especially in sheep. The HI for the two metals for the consumption of offal, especially from aged animals, is a significant cause for concern. Cattle and sheep are considered accurate biomarkers of human exposure to cadmium and lead in Egypt.

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