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Original Research Article

TOXIC METAL RESIDUES IN NON-EDIBLE ANIMAL BYPRODUCTS

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Abstract: Environmental pollution by heavy metals is a major problem worldwide. Domesticated animals such as cattle and camel share the same environmental conditions like human and they are exposed to heavy metals via different sources. Therefore, these animals are considered as ideal bio-indicators for human exposure to heavy metals. Heavy metals accumulate in the different tissues of the animals. Estimation of toxic metal residues such as arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) in the animal edible tissues had been extensively studied. However, estimation of such toxicants in the non-edible animal byproducts had received little attention. Additionally, non-edible animal byproducts are frequently used in many industries such as animal feed additives and leather fabrication. Therefore, this study was undertaken to estimate the residual concentrations of As, Hg, Pb and Cd in the hair, hides and bones of cattle and camel slaughtered at Zagazig, Abo-Hammad and Belbies cities, Sharkia Governorate, Egypt. Metal-metal correlations were additionally calculated. The achieved results indicated exposure of cattle and camel to high levels of heavy metals, particularly lead and arsenic. Camel had higher concentrations (mg/kg ww) of arsenic compared with cattle particularly in hair (38.57 ± 8.77 and 22.48 ± 1.91 in camel and cattle, respectively). Bone had the highest load of the measured metals among examined samples. For instances, in camel, elemental concentrations (mg/kg ww) in bone were 34.53 ± 6.16 (As), 3.41 ± 0.56 (Hg), 2.76 ± 0.36 (Pb) and 0.11 ± 0.01 (Cd). Samples collected from Zagazig city were highly contaminated compared with other locations. Significant positive correlations were observed between lead - mercury, lead - cadmium and arsenic- mercury (r <0.0001 in each). Contaminated non-edible animal byproducts should be hygienically disposed and avoid its introduction to downstream industries. It is highly recommended to control environmental pollution with heavy metals in Egypt.

Key words: bone; domesticated animals; hair; heavy metals; hide

Introduction

Heavy metals are highly accumulative environmental pollutants that can get entry into the ecosystem via anthropogenic activities and subsequently find their way into human and animal bodies via consumption of contaminated food and water leading to several adverse outcomes (1).

Heavy metals such as cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) are classified as toxic heavy metals. For instances, Cd exposure is linked to kidney damage and skeletal muscle deformities (2). Metals like Pb

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may cross blood brain barrier leading to nervous manifestations in both children and adults (3). Arsenic is reported to cause hyperpigmentation and hyperkeratosis of the skin (4). Mercury can lead to multiple organ damage as it had pulmonary, nervous and cardiac symptoms (5).

Domesticated animals such as cattle and camel are reared for their meat and milk and other non-edible animal byproducts, such as hair, hides, bones, that can be used in many industries such as leather fabrication and as ingredients in the animal feed additives (6). These animals are sharing humans in the same environment and therefore are considered as ideal bio-indicators for human exposure to environmental pollutants like heavy metals (7).

Estimation of heavy metals in the edible tissues of domesticated animals had been extensively studied. However, limited information is available about the residual concentrations of the toxic metals in the non-edible animal byproducts such as hair, hides and bone in Egypt. Therefore, this study was undertaken to estimate the residue levels of four toxic metals including As, Hg, Pb and Cd in three non-edible animal products, namely hair, hides and bone of cattle and camel slaughtered at Zagazig, Abo-Hammad and Belbies cities in Sharkia Governorate, Egypt. Additionally, the inter-metal correlations in the examined samples were calculated. Finally, the public health significance of the examined metals was also discussed.

Material and methods

Guidelines of Zagazig University Animal Ethics Committee were followed during conducting this research. All chemicals were the highest quality available and purchased from Merck, Darmstadt, Germany.

Collection of samples

One hundred and eighty samples were collected randomly and equally from non-edible animal byproducts (hair, hides and bone) of both cattle and camel slaughtered at Zagazig, Abo-Hammad and Belbies cities, Sharkia Governorate, Egypt. The sampling sites are characterized by being rural and the animal farms are usually located far from highways. Samples were collected in the period of March to October 2017. Samples were kept frozen in plastic falcon tubes at -20˚C until the time of heavy metal extraction and measurements.

Preparation of samples

Hair samples were washed using distilled water several times for complete removal of any dirt, dried at room temperature, and then cut into small pieces to be easily digested by the acid mixture in the following step.

Hide samples were brushed under running water to remove any dirt followed by washing using distilled water, then cut into small pieces and kept dried.

Bone samples were washed with distilled water to remove any dirt. After washing, bones were cut into small pieces and then treated with a 30% H_2O_2 solution to remove any traces of flesh, fat and blood followed by another wash with distilled water (7). Then one gram from each sample was heated in a hot air oven at 100°C for 2 h followed by another heating cycle for 2 h after mixing with 5 ml of perchloric acid 70% (8).

Digestion of samples

One gram of each sample was mixed with digestion mixture consisted of 5 ml HNO_3 (65%) and 2 ml perchloric acid 70% (9). The mixture was heated at 50°C for 3 h in the water bath, followed by filtration, and dilution. Samples were kept at room temperature until metal analysis.

Analytical procedures

All analytical procedures were done at the Central Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt. Residues of arsenic and mercury were estimated using hydride generation/cold vapor atomic absorption spectroscopy, however, graphite furnace was used in case of lead and cadmium (Perkin Elmer® PinAAcle™ 900T atomic absorption spectrophotometer) (Shelton, CT, USA).

Quality assurance and quality control

Reference material (fish protein) named DORM-3 purchased from the National Research Council, Canada was used to validating the analytical procedures of heavy metals. The reference material was exposed to the same analytical method as in the field samples, and the tested metals were estimated. Then the obtained concentrations were compared with the metal concentrations loaded on the reference material to obtain the recovery rates. Recovery rates ranged from 80% to 115% for all metals examined. The detection limits (mg/kg) for the analyzed metals were 0.02 for arsenic, 0.01 for mercury, 0.1 for lead and 0.001 for cadmium.

Statistical analysis

Statistical significance was evaluated using one-way analysis of variance (ANOVA) and Tukey-Kramer test with P-value < 0.05 considered to be significant. Pearson Correlation coefficient (*r*) was obtained using SPSS program version (23.0).

Results and discussion

Levels of toxic metals in non-edible animal byproducts

The achieved results in Table (1) showed that arsenic was detected in all examined samples. The residual concentrations of arsenic (mg/kg ww) in hair, hides and bone of cattle were 22.48 ± 1.91, 7.59 ± 0.65 and 16.53 ± 1.47, respectively. These concentrations were 38.57 ± 8.77, 14.56 ± 3.67 and 34.53 ± 6.16 in the examined hair, hides and bone of camel (Table 1). It is clear that, camel had higher arsenic residual concentrations compared with cattle in all examined samples. This may be attributed to the ability of camel to live under low water levels and subsequently concentrates the metals into its different tissues (10). Hair samples had the highest residue levels of arsenic followed by bone and hides in both of cattle and camel. This may be due to the frequent exposure of hair to contaminated dusts and water with arsenic. Presence of high concentrations of arsenic in the hair samples despite being washed during sample preparation indicates accumulation of arsenic in hair due to past exposure and it is reported that hair is the target site for arsenic (11). The recorded concentrations of arsenic in the present study is extremely high when compared with the washed cattle hair pastured in the vicinity of the Glogow copper smelter; West Bengal, India and in antlers of red deer in Poland (12-14). This may reflect the high load of as in the Egyptian environment, probably due to the uncontrolled agricultural and industrial activities.

Mercury was detected in all examined samples, where, its levels in the examined samples of cattle were comparable to those of camel. The load of mercury (mg/kg ww) in hair, hides and bone of cattle were 0.77 ± 0.19, 0.51 ± 0.17 and 3.75 ± 0.52, respectively. While in camel, these concentrations were 0.83 ± 0.19, 0.10 ± 0.04 and 3.41 ± 0.56 in the examined hair, hides and bone, respectively (Table 1). It is obvious that, bone had significantly higher mercury residues compared with hair and the hide. The high mercury concentrations in the bone tissue agreed with that reported in Chinese rhesus monkeys (15). Hair had significantly higher concentrations of mercury compared with that of the hide, particularly in the camel. It is reported that hair is rich in sulfur containing amino acid cysteine, which is a major mercury-binding protein (16).

Table 1: Arsenic and mercury residues (mg/kg ww) in the non-edible animal byproducts of cattle and camel

	Arsenic				Mercury			
	Cattle		Camel		Cattle		Camel	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Hair	12.28 –32.88	22.48 ± 1.91^{a}	4.41 –73.91	38.57 ± 8.77^{a*}	0.04 – 1.71	0.77 ± 0.19^{b}	0.03 – 1.72	0.83 ± 0.19^{b}
Hide	5.47 –12.21	7.59 ± 0.65^{c}	2.13 –36.24	14.56 ± 3.67^{b*}	0.01 – 1.79	0.51 ± 0.17^{b*}	0.01 – 0.33	0.10 ± 0.04^{c}
Bone	10.11 –23.06	16.53 ± 1.47^{b}	9.33 –62.03	34.53 ± 6.16^{a*}	0.84 – 6.77	3.75 ± 0.52^{a}	0.72 – 6.03	3.41 ± 0.56^{a}

a-b-c: Means in the same column carrying different superscript letter are significantly different with each other (Comparisons among different tissues of the same species). Means carrying star mark indicate significant difference between same tissue in cattle and camel (p <0.05).

The lead was detected in all examined samples in the present study. Levels of lead residues were comparable among both cattle and camel. Interestingly, bone had significantly higher lead residues followed by hair and hides, respectively (Table 2). Lead has the tendency to accumulate in the bone tissue (17). The residual concentrations of lead in the bone (mg/kg ww) were 2.46±0.28 and 2.76 ± 0.36 in cattle and camel, respectively, while that in the hair were 0.99±0.25 and 0.86±0.24 in cattle and camel, respectively. Hide had the lowest residues of Pb (0.44±0.11 mg/kg ww) in cattle and (0.58±0.09 mg/kg ww) in camel (Table 2). The results of the current investigation agreed with that reported in washed cattle hair pastured in the vicinity of the Glogow copper smelter and in the hair samples collected from camels in Aswan (12, 18). Lower concentrations were recorded in red deer antler in Poland (14). Lead was detected previously in the edible offal of cattle and sheep collected from the same locations (7). Erythrocytes are considered the target site for lead binding in 99% of the absorbed lead. Therefore, lead found its way to soft tissues (liver and kidney) and to bones with the highest precipitation percentages in the bones (18).

Cadmium was detected in all examined tissues in the present study (Table 2). The residual concentrations of cadmium (mg/kg ww) in the examined samples of cattle were 0.13±0.01 in hair, 0.07±0.01 in hides and 0.21±0.02 in bone. These concentrations were 0.09±0.06, 0.01±0.003 and 0.11±0.01 in the examined hair, hides and bone of camel (Table 2). It is clear that, bones had the highest residual contents of cadmium followed by hair and finally the hide. Cattle bone had the highest cadmium residues among the examined samples, which may be explained by the interactions between cadmium and calcium in the skeletal system (19). However, cadmium has no ability to pass the skin barrier (20). The recorded concentrations of cadmium in the current study were slightly higher than that reported in washed cattle hair pastured in the vicinity of the Glogow copper smelter and in red deer antler bone in Poland (12,14), but lower than that recorded in Kalabsha and Halaiub (18). Similarly, high cadmium residues were recorded in the liver and kidneys of cattle and sheep slaughtered in Zagazig city (7).

Arsenic, mercury, lead and cadmium residues were extremely high in samples collected from Zagazig city compared with Abo-hammad and Belbies (Figure 1A, B, C and D). This may be explained by the increase in the anthropogenic activities and traffic deposition in Zagazig city compared with the rural nature of the other locations. Heavy metal load is positively correlated with the traffic deposition (21). Moreover, the Egyptian population in Sharkia Governorate depends mainly on agricultural and small industrial activities, so, many regions of the Governorate have been contaminated with wastewater and solid wastes (7).

In general, presence of such toxic metal residues in animal byproducts reflects the exposure of the living animals to a vast array of xenobiotics including, heavy metals. Subsequently, this indicates contamination of the environment surrounding animals such as air, food and water with such toxicants. Additionally, contamination of such animal

byproducts with toxic metals may lead to loading of the downstream products such as animal feeds and leather products with such toxic metals. Then these metals can find their way to other animals or humans using such contaminated products.

Table 2: Lead and cadmium residues (mg/kg ww) in the non-edible animal byproducts of cattle and camel

	Lead				Cadmium			
	Cattle		Camel		Cattle		Camel	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Hair	0.06 –1.94	0.99 ± 0.25^{b}	0.05 –2.08	0.86 ± 0.24^{b}	0.04 – 0.18	0.13 ± 0.01^{b}	0.01 – 0.64	0.09 ± 0.06^{a}
Hide	0.05 –0.94	0.44 ± 0.11^{c}	0.01 –1.16	0.58 ± 0.09^{b}	0.04 – 0.15	$0.07 \pm 0.01^{c*}$	0.001 – 0.04	0.01 ± 0.003^{b}
Bone	0.72 –3.66	2.46 ± 0.28^{a}	1.27 –4.26	2.76 ± 0.36^{a}	0.13 – 0.32	$0.21 \pm 0.02^{a*}$	0.05 – 0.17	0.11 ± 0.01^{a}

a-b-c: Means in the same column carrying different superscript letter are significantly different with each other (Comparisons among different tissues of the same species). Means carrying star mark indicate significant difference between same tissue in cattle and camel (p <0.05).

Table 3: Significant Pearson correlation's coefficient values between toxic metals in the examined non-edible byproducts of cattle and camel

Cattle			Camel		
Metal	***r*** ***p value***	**Tissue**	**Metal**	***r*** ***p value***	**tissue**
Pb-Cd	0.949 <0.0001	bone	Pb-Hg	0.947 <0.0001	bone
Pb-Cd	0.914 <0.0001	hide	Pb-Hg	0.847 <0.0001	hide
Pb-Hg	0.901 <0.0001	hair	Pb-Hg	0.965 <0.0001	hair
As-Hg	0.906 <0.0001	bone	As-Cd	0.887 <0.0001	bone
As-Hg	0.988 <0.0001	hide	As-Cd	0.800 <0.0001	hide
As-Hg	0.944 <0.0001	hair	As-Cd	0.539 0.0043	hair

Values in the table are the significant correlations at p <0.05. r: Pearson correlation coefficient; p: probability; Pb: lead; Cd: cadmium; As: arsenic; Hg: mercury

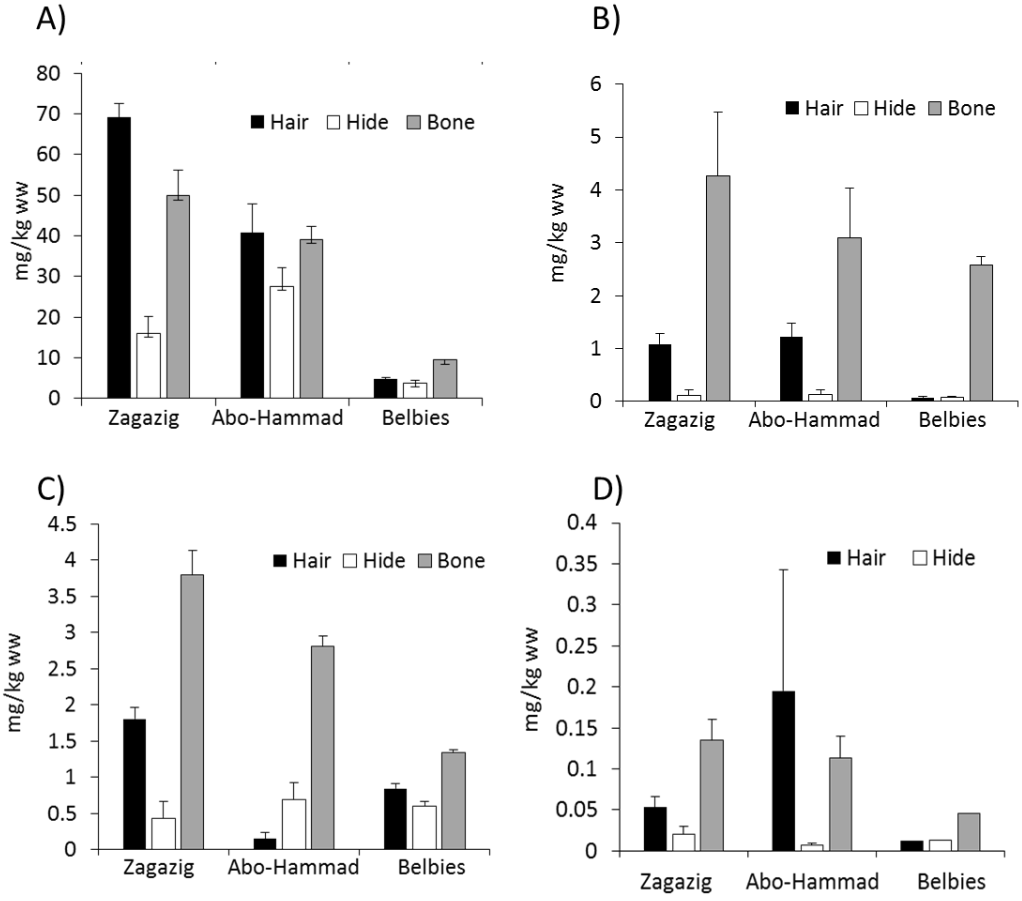

Figure 1: Toxic metal residues in non-edible animal byproducts of cattle and camel in different localities at Sharkia Governorate, Egypt (2017). A) Arsenic, B) mercury, C) lead and D) cadmium residues (mg/kg ww) in hair, hide and bone of cattle and camel from Zagazig, Abo-Hammad and Belbies. Values represent mean ± SE (n = 90 from each animal species divided as 10 samples from each byproduct/ each animal species/each location)

Correlations among metals in the non-edible animal byproducts

As a method of metal bio-detoxification, interactions between toxic and essential metals take place in the animal body. For instances, cadmium and lead absorption increases by iron deficiency. Selenium can protect against mercury-induced cytotoxicity. Lead replaces zinc on haeme enzymes (21). The results described in Table (3) showed the significant positive correlations among the examined metals in the non-edible byproducts of cattle and camel. It is clear that there were positive correlations between lead-cadmium (p <0.0001) in bone and hides of cattle; lead-mercury (p <0.0001) in all examined tissues of camel; arsenic-mercury in cattle tissues and arsenic-cadmium (p <0.0001) in tissues of camel. Nearly similar correlations were detected in the edible tissues of cattle and sheep (7) and in the non-edible tissues of camels (18). Future studies are still needed to estimate the essential trace elements such as iron and selenium to have comprehensive image about correlations among metals in such animal byproducts. Furthermore, levels of toxic metals in the blood should be measured in future studies.

Conclusion

The attained results of the present study declared that non-edible animal byproducts of cattle and camel collected from Sharkia Governorate, Egypt were contaminated with toxic metals including arsenic, mercury, lead and cadmium. Therefore, such contaminated byproducts should be hygienically disposed and

not introduced into manufacturing purposes. In addition, strict control measures should be adopted to reduce the exposure of animal and human to such toxicants.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Cheng S. Heavy metal pollution in China: origin, pattern and control. Environ Sci Pollut Res Int 2003; 10: 192–8.

2. Järup L. Hazards of heavy metal contamination. Br Med Bull 2003; 68: 167–82.

3. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic Effects and Biomarkers of Lead Exposure: A Review. Rev Environ Health 2009; 24: 15–45.

4. Tondel M, Rahman M, Magnuson A, Chowdhury IA, Faruquee MH, et al. The relationship of arsenic levels in drinking water and the prevalence rate of skin lesions in Bangladesh. Environ Health Perspect 1999; 10: 727–9.

5. Graeme KA, Pollack CV Jr. Heavy metal toxicity, Part I: arsenic and mercury. J Emerg Med 1998; 16: 45–56.

6. Alao BO, Falowo AB, Chulayo A, Muchenje V. The Potential of Animal By-Products in Food Systems: Production, Prospects and Challenges. Sustainability 2017; 9:1089.

7. Darwish WS, Hussein MA, El-Desoky KI, Ikenaka Y, Nakayama S, et al. Incidence and public health risk assessment of toxic metal residues (cadmium and lead) in Egyptian cattle and sheep meats. Int Food Res J 2015; 22: 1719–26.

8. Yamada M, Tohno S, Tohno Y, Minami T, Ichii M, Okazaki Y. Accumulation of mercury in excavated bones of two natives in Japan. Sci Total Environ. 1995; 162(2-3): 253–6.

9. Alqadami AA, Khan MA, Otero M, Siddiqui MR, Jeon B et al. A magnetic nanocomposite produced from camel bones for an efficient adsorption of toxic metals from water. J Cleaner Prod 2018; 178: 293–304.

10. Soliman MK. Functional anatomical adaptations of dromedary (Camelus Dromedaries) and ecological evolutionary impacts in KSA. International Conference on Plant, Marine and Environmental Sciences (PMES-2015) Kuala Lumpur (Malaysia). 2015.

11. Yáñez J, Fierro V, Mansilla H, Figueroa L, Cornejo L et al. Arsenic speciation in human hair: a new perspective for epidemiological assessment in chronic arsenicism. J Environ Monit 2005; 7: 1335–41.

12. Rogowska KA, Monkiewicz J, Grosicki A. Lead, cadmium, arsenic, copper, and zinc contents in the hair of cattle living in the area contaminated by a copper smelter in 2006 – 2008. Bull Vet Inst Pulawy 2009; 53:703–6.

13. Rana T, Bera AK, Das S, Bhattacharya D, Pan D et al. Subclinical arsenicosis in cattle in arsenic endemic area of West Bengal, India. Toxicol Ind Health 2014; 30: 328–35.

14. Giżejewska A, Szkoda J, Nawrocka A, Żmudzki J, Giżejewski Z. Can red deer antlers be used as an indicator of environmental and edible tissues' trace element contamination. Environ Sci Pollut Res Int 2017; 24: 11630–8.

15. Lee JI, Jung WY, Lee G, Kim MS, Kim YS et al. Heavy metal concentrations in hair of newly imported China-origin rhesus macaques (*Macaca mulatta*). Lab Anim Res 2012; 28: 151–4.

16. Nuttall KL. Interpreting hair mercury levels in individual patients. Ann Clin Lab Sci 2006; 36(3): 248–61.

17. Rashed MN, Soltan ME. Animal hair as biological indicator for heavy metal pollution in urban and rural areas. Environ Monit Assess 2005; 110: 41–53.

18. Markowitz ME, Shen XM. Assessment of bone lead during pregnancy: a pilot study. Environ Res 2001; 85: 83–9.

19. Keil DE, Berger-Ritchie J, McMillin GA. Testing for toxic elements: A focus on arsenic, cadmium, lead, and mercury. Lab Medicine 2011; 42: 735–42.

20. Goyer RA. Toxic and essential metal interactions. Annu Rev Nutr 1997; 17: 37–50.

21. Shabbaj II, Alghamdi MA, Shamy M, Hassan SK, Alsharif MM, et al. Risk assessment and implication of human exposure to road dust heavy metals in Jeddah, Saudi Arabia. Int J Environ Res Public Health 2017; 15: 36.