Some Heavy Metal Contents of Various Slaughtered Cattle Tissues in Sivas-Turkey

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Abstract: As a result of environmental pollution and food chain, heavy metals may accumulate in human or animal bodies. Toxic metals cause inhibition on chemical and enzyme reactions in cells. Therefore, a negative situation forms in organs and tissues due to their toxic effects. The examination of metal pollution in foods and environment facilitates to monitor their harmful effects on human health. In the proposed study, the concentrations of toxic metals in tissue samples of animal obtained from a local farm were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following micro-wave digestion. Five different tissue samples were studied including lung, liver, kidney, muscle, and brain. Metals were showed various distribution in the different organs. The highest concentration of Al in the lung, Cu, Mn, and Mo in the liver Cr, As and Se in kidney, and V in kidney and brain were found. The mean values obtained for kidney, liver, lung, muscle and brain of cattle tissues were: 2.40, 2.44, 3.73, 2,90, 3.07 mg/kg Al; 0.35, 0.26, 0.29, 0.27, 0.36 mg/kg V; 3.95, 7.00, 0.76, 0.45, 1.19 mg/kg Mn; 15.82, 280.86, 7.94, 3.85, 10.64 mg/kg Cu; 1.87, 4.25, 1.19, 0.15, 0.28 Mo; 0.47, 0.14, 0.10, 0.07, 0.04 mg/kg As; 0.47, 0.33, 0.41, 0.39, 0.43 mg/kg Cr; 4.38, 1.38, 0.82, 0.60, 0.56 mg/kg Se respectively. Certified reference material (NIST CRM 2976 muscle tissue) was analyzed for accuracy of method. This results are good agreement (≥95 %) with the certified values.

Keywords: Accumulation of heavy metals, Cattle tissue, ICP-MS.

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INTRODUCTION

In fact, the heavy metal term is used as a physical property of the metals whose density is greater than 5 g/cm^3. Heavy metals are classified as vital and non-vital metals according to their degree of participating to biological activities (1).Those that are defined as essential should be present in the organism at certain concentrations. In addition, since they participate biological reactions, these metals should regularly be taken through food (2). For example, copper is an indispensable part of red blood cells, as well as many oxidation and reduction reactions in humans and animals. However, it should be noted that high amount of heavy metals is toxic. On the other hand, non-essential heavy metals (Pb, Cd, Hg, *etc*.) may cause health problems by damaging biologic structure even at the lowest concentrations (3-4). Heavy metals have a risk potential for ecologic system and human health because, unlike organic polluter, they are not degraded, and they are accumulated in the food chain. Heavy metal pollution is one of the significant ecologic problems in many rapidly developing countries (5-7).

Animals and humans take heavy metals released into the air from industrial activities through inhalation, in addition, they join the food chain when reaching the earth. Heavy metals, may also have harmful effects on living organisms through wastewater mixed in drinking water or through the pollination of the particles contaminated with heavy metals (8-15).

Heavy metals that animals orally get from their forage and water, or they receive from their surroundings accumulate in the tissue of their organs, such as liver, kidney, which is widely consumed by humans as a source of animal protein in the overall world. Consumers have no knowledge of the quantity of the heavy metal in the content of these products and the risk they carry for the health (16, 17). Therefore, monitoring the heavy metal concentration present in the tissues of this organ is significant regarding the control of the biochemical processes and ecosystem. In addition, heavy metal content in organisms provides insight into the risk of environmental pollution (18-21).

This study was evaluated the concentrations of some heavy metals in the kidney, liver, lung, muscle, and brain of slaughtered cattle from Sivas, Turkey

MATERIALS AND METHODS

Trace metal grade reagents were used. Multi-element standard solutions were purchased from BDH Chemicals. A certified reference material (muscle tissue) was used to check the accuracy of the method.

Instrumentation

Studies were carried out by using a Thermo Scientific ICAP-Q ICP-MS instrument. The instrument was equipped with PFA-ST MicroFlow nebulizer and cyclonic quartz spray chamber offering exceptional sensitivity for analysis of samples with small volumes. Microwave Digestion System (CEM MARS 6) was used for digestion tissue samples. ICP-MS operating conditions summarized in Table 1.

Table 1: ICP-MS operating conditions.

RF power (kW)	1.55
Nebulizer	PFA-ST MicroFlow Nebulizer
Spray chamber	Cyclonic Quartz
Plasma Ar (L min^{-1})	14
Auxiliary Ar (L min^{-1})	0.80
Nebulizer Ar (L min^{-1})	1.0
Sample uptake/mL min^{-1}	0.5
Scanning mode	Peak hopping
Dwell time (s)	0.01

Sampling

Tissues of cattle organs were collected from one of the biggest farms in Sivas. The animals used in the study were two years old and healthy. Fifteen animals were studied as sample throughout a year. All samples were kept in plastic bags and immediately transported to the laboratory. The samples were washed with deionized water, cut into small pieces, and dried at 60 °C. Then, they were homogenized and stored at 4 °C until analysis**.**

Microwave Assisted Digestion Procedures

Microwave vessels (PTFE) were cleaned using 10 mL of concentrated HNO_3, heated for 15 min at 180 °C (800 W), and then rinsed with ultrapure water heated for 15 min at 180 °C before each digestion. All samples were accurately weighted as 0.50 g, transferred directly into microwave vessels, and added 5 mL of concentrated HNO_3. The blank solutions, which contain 5 mL of concentrated HNO_3, were also prepared during the analysis of each sample. The analysis of the sample and blank solutions were performed in three replicates and were diluted 10 times with deionized water before analyzed by ICP-MS. The digestion program was chosen in agreement with manufacturer's recommendations, and earlier studies on microwave assisted digestion optimization. Digestion procedure as summarized in Table 2.

Table 2: Operating conditions for microwave digestion methods.

Ramp Time	20 min
Hold Time	15 min
Temperature	200 °C
Power	800 W

RESULTS AND DISCUSSION

The detection limits, calibration equations were summarized for all elements in Table 3. The calibration curves for all elements were linear within the range from 0.5 to 50 µg/L. The detection and quantification limits were calculated over 10 measurements of the blank for microwave procedure, as 3s/b and 10s/b, respectively.

Table 3. The detection limits (LOD, µg/L) and typical calibration curves **(n=10).**

Element	Isotope	LOD (µg/L)	LOQ (µg/L)	Calibration Equation	R^2
Al	27	0.233	**0.777**	y = 22337x + 15105	0.9974
V	51	0.090	**0.300**	y = 47959x + 61779	0.9974
Mn	55	0.032	**0.107**	y = 67950x + 72611	0.9986
Co	59	0.072	**0.240**	y = 50251x + 24863	0.9995
Ni	60	0.070	**0.233**	y = 11444x + 6706	0.9951
Cu	65	0.236	**0.787**	y = 12050x + 27863	0.9947
As	75	0.057	**0.190**	y = 5967x + 8573	0.9975
Mo	95	0.024	**0.080**	y = 19728x + 3733	0.9994
Cr	52	0.012	**0.040**	y = 110865x -70227	0.9976
Se	77	0.07	**0.233**	y = 1152x +3367	0.9993

The concentrations of heavy metals in the kidney, lung, liver, brain and muscle samples were determined by ICP-MS. All results were presented as miligrams per kilograms. Mean concentrations of elements (mg/kg dry weight) in tissue of cattle were summurized in Table 2. The accuracy of the method was evaluated by comparing with certified values of NIST SRM 2976. The achieved results were in good agreement with certified values. The results for this study were given in Table 3.

Table 4: The content of heavy metals in the liver, kidney, muscle, and brain in cattle in Sivas, Turkey (mg/kg dry weight, n=15).

		Al	V	Mn	Cu	As	Mo	Cr	Se
Kidney	Minimum	1.46	0.24	5.23	8.19	0.36	1.08	0.30	3.14
	Maximum	3.11	0.53	2.73	19.46	0.57	2.72	0.67	6.01
	Average	2.40	0.35	3.95	15.82	0.47	1.87	0.47	4.38
	Sd	0.5	0.09	0.82	3.58	0.07	0.42	0.13	0.88
Liver	Minimum	1.23	0.21	1.15	205.28	0.09	2.5	0.24	0.79
	Maximum	5.69	0.37	8.80	386.42	0.2	4.88	0.5	2.32
	Average	2.44	0.26	7.00	280.86	0.14	4.25	0.33	1.38
	Sd	1.06	0.04	1.8	98.49	0.03	0.64	0.07	0.45
Lung	Minimum	1.58	0.21	0.48	3.01	0.07	0.2	0.26	0.30
	Maximum	5.02	0.4	1.32	16.75	0.14	2.00	0.63	1.58
	Average	3.73	0.29	0.76	7.94	0.1	1.19	0.41	0.82
	Sd	1.29	0.07	0.28	4.40	0.02	0.58	0.17	0.34
Muscle	Minimum	1.94	0.20	0.22	1.59	0.05	0.08	0.25	0.20
	Maximum	4.79	0.43	0.85	6.90	0.11	0.28	0.67	1.10
	Average	2.90	0.27	0.45	3.85	0.07	0.15	0.39	0.60
	Sd	0.90	0.06	0.17	1.64	0.02	0.05	0.10	0.25
Brain	Minimum	1.69	0.22	0.85	5.66	0.02	0.19	0.12	0.27
	Maximum	5.88	0.47	1.51	14.15	0.06	0.41	0.61	0.76
	Average	3.07	0.36	1.19	10.64	0.04	0.28	0.43	0.56
	Sd	1.52	0.07	0.29	2.85	0.01	0.08	0.13	0.17

Sd: standard deviation

Table 5. Trace element concentrations in certified reference material (NIST SRM 2976 muscle tissue) n=3.

Element	Found (mg/kg)	Certified value (mg/kg)	Recovery (%)
Al	128.0±6.7	134±34	96
Mn	31.6± 1.3	33 ± 2	96
Cu	3.8±0.2	4.02±0.33	95
As	12.9± 0.6	13.3± 1.8	97
Cr	0.46± 0.01	0.50 ± 0.16	92
Se	1.72± 0.03	1.80 ± 0.15	95

The results show Cu, Mn and Mo to concentrate primarily in the liver; As, V and Se in the kidney, Al in the lung; V in the lung and brain.

Cu, Mn and Cr are an essential trace element that is required for the metabolism. These elements' both the deficiency and excess can cause health problems. Deficiency of Cu can lead to anemia and excess of Cu causes changes in the color of skin and hair. If an excessive amount of Cu is taken, the kidney and liver can be damaged. Even the accumulation of excessive Cu in the liver can cause cirrhosis or hepatitis. Acute poisoning in human for Cu is 100 mg/kg as World Health Organization (WHO). According to WHO, copper and manganese are taken 3 and 2-9 mg per daily respectively Mn and Cu values have been reported as 15.3-1.2, 156-28 mg/kg in the literature, respectively. The highest Mn, Cu, Mo, and concentration were found to be 7.00, 280.86, 4.25 $mg.kg^{-1}$respectively. Our Mn values are in agreement with reported data from the literature. However, our Cu values are higher than literature values and permissible values of WHO.

Mo values in liver, kidney, and lung are 4.25, 1.87, 1.19 mg/kg respectively. Mo values in muscle and brain are lower compared to other tissues. In the literature could not be reached to about Mo levels in animal tissues.

The chromium levels were found 0.12-0.61 mg/kg in our study. These results are lower compared with literature values and Food and Agriculture Organization (FAO)/WHO limit value which is 1.0 mg/kg (22, 23).

The Al concentration in the lung is relatively higher, compared with the concentration in other tissues. To the best of acknowledging our accumulation of Al in cattle tissue is no information. Selenium is a component of the cytosol enzyme which is glutathione peroxidase and has an important role in intracellular oxidation-reduction reactions. The absorbed Se in the internal organs decreased in the following order: kidney, liver, lung, muscle, and brain in our study. The highest concentration for Se is 4.38 mg/kg in the kidney.

The levels of V is ranged between 0.26-0.36 mg/kg. This range is narrow when it is compared with other elements in tissue. Our study results showed that there was no significant difference in the concentration of V in all of the tissue of cattle.

As is a non-essential element. It is found that the arsenic accumulates the hair and nail primarily and also liver and kidney in mammals. Most body tissues contain less than 0.3–147 μg/g (dry weight) except for hair, nail, and teeth. As levels in our study ranged between 0.47-0.04 mg/kg and highest concentration in the kidney as 0.47 mg/kg. Our As values are in agreement with reported data from the literature.

Distribution of these elements in the organs are related to this specific physiological roles of elements and their relative abundance in intracellular ligands able to bind metals.

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