Bull. Environ. Contam. Toxicol. (1998) 60:531-537

Bulletin of Environmental Contamination and Toxicology

Trace Metals in Moose (*Alces alces*) Liver

R. G. Treble, T. S. Thompson

Saskatchewan Health, Provincial Laboratory, Environmental Toxicology Section, 3211 Albert Street, Regina, Saskatchewan, Canada S4S 5W6

Received: 20 October 1997/Accepted: 29 January 1998

Elements may be classified as being either toxic or essential based upon their role in various biochemical processes. Generally speaking, toxic elements such as lead, mercury and arsenic are those which produce deleterious effects as a result of either chronic or acute exposure. Conversely, essential elements such as calcium and iron are those which are required to sustain proper functioning of specific biochemical processes. In many cases, deficiencies of these elements results in health disorders.

Elemental concentrations in biological fluids such as blood or urine often do not indicate the degree of accumulation by the body. One method of estimating the body burden of trace elements is to analyze the tissue of organs such as the liver or kidneys. Typically spectroscopic techniques such as graphite furnace atomic absorption spectroscopy (GFAAS), flame AAS, and inductively coupled plasma (ICP) spectroscopy have been utilized for the determination of trace elements in biological tissue.

There have been numerous studies in which various elements have been determined in the organs of animals. Liver, kidney cortex, and pancreas samples were taken from immature foals raised near a zinc smelter and analyzed for zinc, cadmium, copper, lead, magnesium, and calcium (Kowalczyk 1986). Only the zinc and cadmium levels were significantly elevated in all three tissue types. Unlike zinc, the concentrations of cadmium directly correlated with age in all tissues examined. Martin (1989) used GFAAS with Zeeman background correction for the determination of selenium in bovine liver. Flame AAS was used for the determination of copper and iron in various animal liver tissue samples (Vidal 1990). Flame AAS was also used for the determination of copper, iron, manganese, and zinc in bovine liver (Farah and Sneddon 1993). Smith-Hieftje background correction was applied for all analyses. Benemariya (1993) studied the levels of zinc, copper, and selenium in miscellaneous organs of cows and goats from Burundi, Africa. The liver was found to be the organ which accumulated the highest levels of copper.

While many studies of biological tissues involve an acid digestion procedure in

Correspondence to: T. S. Thompson

which a homogeneous digested solution was analyzed, solid sampling techniques have been used in some applications. Chakrabarti (1987) compared various atomization techniques for use with solid sampling the GFAAS determination of lead in bovine liver. Untreated bovine livers were sampled by autoprobe and directly analyzed by Zeeman GFAAS for lead and cadmium (Luecker 1992). Lead and cadmium have also been determined in the liver of mallard ducks (Luecker 1993a) and horses (Luecker 1993b) by analyzing solid samples using graphite furnace Zeeman AAS. A high pressure homogenizer was utilized for preparing a slurry of frozen tissue (cervine liver or kidney) in ethanol-water which was subsequently analyzed for cadmium, copper and lead using GFAAS (Tan 1996). This analytical procedure was found to compare favourably with a method involving conventional acid digestion and analysis by ICP-MS. The method was limited by the contamination of the sample by lead and copper from the homogenizer.

In the work described herein, GFAAS techniques were employed for the determination of copper, lead, and manganese in moose liver tissue. Flame AAS was used to analyze zinc levels in the same digest samples.

MATERIALS AND METHODS

Portions of moose liver tissue specimens from animals which were hunted in the central western region of the province of Saskatchewan were obtained from a wildlife resource officer. All animals were hunted in the approximate vicinity of the towns of Carrot River and Hudson Bay, near the border shared with the province of Manitoba (see figure 1). Approximately 2 to 4 g of wet liver tissue was weighed into a 25-mL round-bottomed tube. The liver was gently dried at 60 °C for 90 hours and the resulting dry weight was recorded. Randomly selected samples were processed in duplicate to verify the precision afforded by the employed methodology. The specimens were allowed to digest in 5 mL of ultrapure concentrated nitric acid (J.T. Basker Inc., Phillipsburg, NJ) for a period of 72 hours at room temperature. The digested liver samples were diluted as necessary to fall within the normal working analytical range of concentration for each element (i.e., quantitation was performed by interpolation of calibration curves). All reagent blanks were prepared and treated in exactly the same manner. As a further quality control check, a standard reference material (SRM 1577a) consisting of lyophilized bovine liver, purchased from the National Institute of Standards and Technology (Gaithersburg, MD), was analyzed along with the liver samples. This material has certified values for all of the analytes determined in this study. Two portions of the SRM liver, each weighing approximately 0.5 g, were processed with the moose liver samples.

A Varian SpectrAA-300Z atomic absorption spectrometer (Victoria, Australia) equipped with a graphite furnace and Zeeman background correction system was used in conjunction with a programmable Varian autosampler. Individual

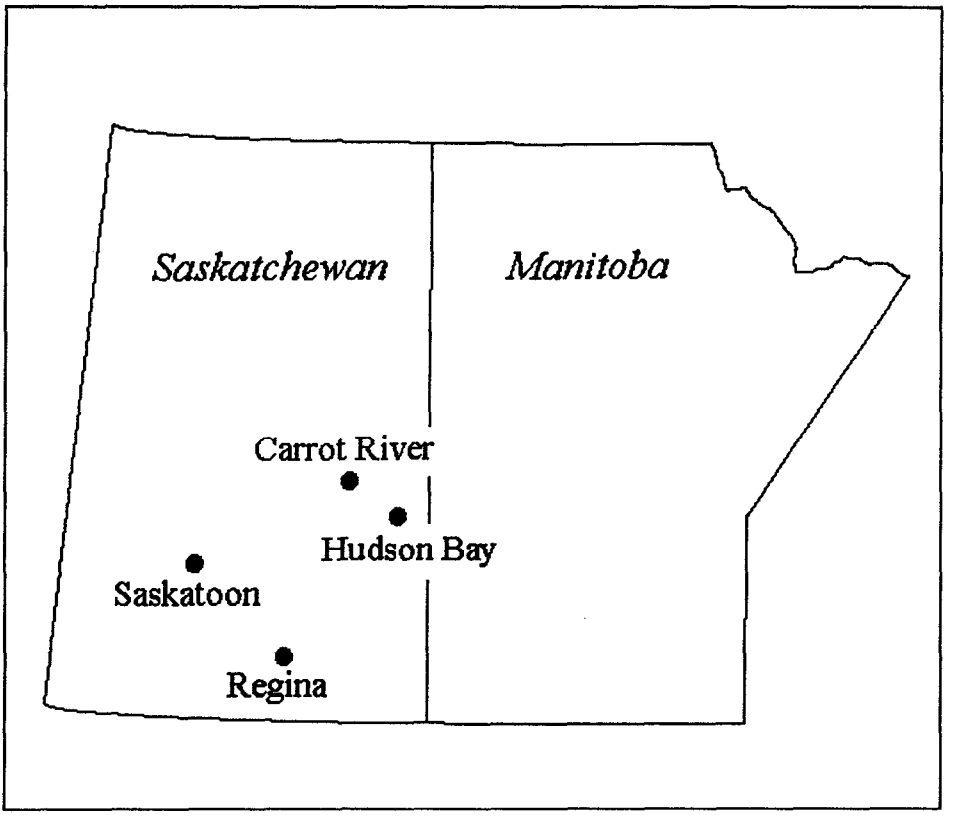

Figure 1. Map of Saskatchewan and Manitoba, Canada.

hollow cathode lamps were employed for the determination of lead, manganese, and copper. Details regarding the conditions of the radiation source for the analysis of each of these 3 elements are given in Table 1. Pyrolytically-coated partition graphite tubes purchased from Varian were used for all analyses carried out on the GFAAS. The graphite furnace programs utilized for each analysis are shown in Table 2. Extra steps were employed at the termination of the atomization furnace program to allow the graphite tube to cool slowly to 40 °C and thus prevent spattering of the subsequent injection. Along with this modification, an extended dispensing rate (with heated inject, 40 °C) was used to ensure that all the dispensed liquid would be slowly introduced to be contained within the platform cavity for subsequent temperature programming. The automatic sampler was programmed to deliver 5 mL of solution to the graphite tube.

A Varian SpectrAA-20 flame atomic absorption spectrometer (Victoria, Australia) equipped with a deuterium background correction system was employed for the determination of total zinc. The zinc hollow cathode lamp operating information is given in Table 1.

Table 1. Radiation source conditions for AAS analyses.

	Cu	Mn	Pb	Zn
wavelength (nm)	324.8	279.5	283.3	213.9
slit width (nm)	0.5	0.2	0.5	1.0
current (ma)	4	5	5	5

Table 2. Graphite furnace temperature programming profiles.

Element	Step No.	Temp. (°C)	Ramp Time (s)	Hold Time (s)	Argon Gas Flow (L/min)
Cu	1	120	30	10	3.0
	2	700	30	10	3.0
	3	2600	1	7	0.0
	4	40	13	2	3.0
Mn	1	110	35	15	3.0
	2	650	35	20	3.0
	3	2500	1	67	0.0
	4	40	10	4	3.0
Pb	1	120	65	20	3.0
	2	400	15	7	3.0
	3	2600	1	7	0.0
	4	40	13	2	3.0

RESULTS AND DISCUSSION

Liver tissue samples from a nine moose were dried, subjected to acid digestion, and subsequently analyzed for copper, manganese, and lead using GFAAS and zinc using flame AAS. The hepatic metal concentrations are summarized in Table 3 along with a brief description of the location in which the animal was hunted.

The levels of copper, manganese, and lead were found to be relatively consistent for all of the samples which were analyzed. In fact, the percent relative standard deviations for these elements were calculated to be 39, 52, and 28%, respectively. The hepatic zinc concentrations were found to range from 16.9 to 44.8 µg/g in 8 of the samples with one sample having a much higher level of zinc (204 µg/g). Excluding this elevated sample, the average zinc concentration was calculated to be 28.0 µg/g with a percent relative standard deviation of 29%.

Table 3. Trace metals in moose liver.

DESCRIPTION	Cu (µg/g)	Mn (µg/g)	Pb (µg/g)	Zn (µg/g)
bull moose (Smoking Tent Creek; 35 km southeast of Hudson Bay)	41.7	2.57	0.09	20.3
cow moose (Smoking Tent Creek; 35 km southeast of Hudson Bay)	39.2	2.77	0.08	24.2
cow moose (Hudson Bay)	36.9	2.13	0.08	16.9
cow moose (Green Bush Creek; 20 km west of Hudson Bay)	82.7	2.07	0.08	27.4
bull moose (south of Hudson Bay)	14.1	2.77	0.11	25.5
cow moose (south of Erwood; 15 km east of Hudson Bay)	44.7	3.70	0.17	34.6
cow moose (Parr Hill; 50 km southeast of Hudson Bay)	49.4	2.53	0.10	44.8
cow moose (Dagg Creek; 20 km south of Hudson Bay)	70.8	7.93	0.09	204
cow moose (Carrot River)	47.2	2.83	0.08	30.5
AVERAGE	47.4	3.26	0.098	28.0*
STANDARD DEVIATION	18.6	1.71	0.027	8.2*

* Excluding sample having Zn concentration of 204 µg/g.

The use of a standard reference material for which a certified analyte concentration has been established provides an excellent means of verifying the accuracy of the analytical procedure which has been employed. Based upon the duplicate analyses which were performed on the bovine liver SRM (see Table 4) processed along with the moose liver samples, the analytical procedure appears to provide results which are both accurate and precise. Analysis of 2 portions of the SRM liver yielded results which were within the acceptable range of the certified trace metal concentrations.

It should also be recognized that background correction is necessary to determine trace elements in complex matrices such as liver digest samples. Zeeman background correction was employed in all GFAAS analyses. The Zeeman correction technique eliminates background interferences including molecular absorption over the complete analytical wavelength. Utilizing this feature, even extremely low levels of lead could successfully be determined in the lyophilized bovine liver SRM. Deuterium background correction was employed for the flame AAS analysis of zinc.

Table 4. Analysis of trace metals in SRM 1577a lyophilized bovine liver.

Element	Certified Concentration (µg/g)*	GFAAS & Flame AAS Results (µg/g)
Cu	158 ± 7	162, 164
Mn	9.9 ± 0.8	9.8, 9.4
Pb	0.135 ± 0.015	0.142, 0.132
Zn	123 ± 8	118, 117

* Based on certificate of analysis for SRM 1577a

Acknowledgments. The authors gratefully acknowledge the technical expertise and advice provided by Dr. Daniel Paschal (Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA) concerning the sample preparation procedure.

REFERENCES

Benemariya H, Robberecht H, Deelstra H (1993) Zinc, copper, and selenium in milk and organs of cow and goat from Burundi, Africa. Sci Total Environ 128: 83-98

Chakrabarti CL, Karwowska R, Hollebone BR, Johnson PM (1987) Direct determination of lead in bovine liver by solid sampling with graphite furnace atomic absorption spectrometry - a comparison of tube wall atomization, platform atomization, and probe atomization. Spectrochim Acta Part B 42B: 1217-1225

Farah KS, Sneddon J (1993) Simultaneous determination of copper, iron, manganese and zinc in bovine liver and estuarine sediment using flame atomic absorption spectrometry with background correction. Anal Lett 26:709-719

Kowalczyk DF, Gunson DE, Shoop CR, Ramberg CF Jr (1986) The effects of natural exposure to high levels of zinc and cadmium in the immature pony as a function of age. Environ Res 40: 285-300

Luecker E (1992) Solid sampling analysis by means of autoprobe-GFAAS. Part II: direct determination of lead and cadmium in untreated bovine livers. Fresenius' J Anal Chem 343: 386-390

Luecker E, Gerbig C, Kreuzer W (1993a) Distribution of lead and cadmium in the liver of the mallard - direct determination by means of solid sampling ZAAS. Fresenius' J Anal Chem 346: 1062-1067

Luecker E, Meuthen J, Kreuzer W (1993b) Distribution of lead and cadmium in equine liver - direct determination by means of solid sampling ZAAS.

Fresenius' J Anal Chem 346: 1068-1071
Martin CK, Williams JC (1989) Determination of selenium in bovine liver by Zeeman-effect atomic absorption sbectrometry using a palladium-copper chemical modifier. J Anal At Spectrom 4: 691-695
Tan Y, Marshall WD, Blais JS (1996) Slurry preparation by high-pressure homogenization for cadmium, copper and lead determination in cervine liver and kidney by electrothermal atomic absorption spectrometry. Analyst 121: 483-488
Vidal MT, Lizondo F, De la Guardia M (1990) Determination of copper and iron in animal liver samples by flame atomic absorption spectroscopy. At Spectrosc 11: 75-77