S. Afr. J. Chem., 2012, **65**, 159–164,
<http://journals.sabinet.co.za/sajchem/>.

Assessment of Levels of V, Cr, Mn, Sr, Cd, Pb and U in Bovine Meat

Abayneh A. Ambushe[a], Mokgadi M. Hlongwane[a], Robert I. McCrindle[a,*] and Cheryl M.E. McCrindle[b]

[a]*Department of Chemistry, Tshwane University of Technology, P.O. Box 56208, Arcadia, 0007, South Africa.*

[b]*School of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Pretoria, 0001, South Africa.*

Received 26 July 2011, Revized 18 May 2012, Accepted 25 June 2012.

ABSTRACT

Pollution of the environment with heavy metals can be a serious problem. In South Africa, particularly, there are many sources of heavy metals, often due to smelter and mining activities. This has led to toxic metals in the environment that directly affect air, water and food. The presence of heavy metal residues in foodstuffs is potentially hazardous to humans and animals. Heavy metals accumulate in certain organs, particularly in the liver and kidney. The objective of this study was to determine the levels of V, Cr, Mn, Sr, Cd, Pb and U in bovine organs and tissues obtained from polluted areas of North West Province, South Africa. Bovine liver, kidney, muscle, fat and bone samples were freeze-dried, homogenized and mineralized using a microwave-assisted digestion system. The levels were quantified using dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS). A bovine muscle (NIST-RM 8414) and bovine liver, reference material (NIST-RM 1577b), were also analyzed and results agreed with certified values. The study revealed accumulation of Sr in bone, Cd, Pb and U in kidney and bone, Mn in liver and, V and Cr in kidney tissues of cattle.

KEYWORDS

Bovine meat, heavy metals, DRC-ICP-MS.

1. Introduction

Environmental contamination of food is becoming an increasingly important aspect of food safety. Heavy metal residues in food of animal origin are directly related to human illnesses. Yet it is difficult to classify trace metals into essential and toxic groups, although it is well known that an essential metal becomes toxic at high intakes.[1,2]

Meat is an important source of a wide range of essential trace metals for humans, but may also carry toxic metals as residues. Contamination with heavy metals is a serious threat, not only because of their toxicity but also because of bioaccumulation in the food chain.[3] Kidney and liver are the tissues and organs that have a propinquity to bioaccumulate toxic metals such as As, Cd, Hg and Pb.[4] The residues measured in these animal organs may also indicate the degree of pollution of the grazing area and drinking water.[5,6] These organs can, however, also serve as a rich source of essential microelements (notably Fe, Cu, Zn and Se) in the human diet.[7] Kidney and liver are low in cost and are a component of some traditional South African diets, thus toxic residues can affect those with low incomes who may not have access to medical care.

Concentrations of heavy metals in air, water, soils and sediments have been increasing over the last decades, both in urban and periurban areas.[8,9] Heavy metals can be transferred to animals through direct exposure, polluted water and crops irrigated with polluted water.[6,10] In 2003, environmental heavy metal contamination from industrial activities in China, was implicated in animal mortalities. This resulted in major economic losses to local farmers.[11] In South Africa, the public health implications of farming cattle in areas with high background levels of V were originally reported by McCrindle *et al*.[12] and further investigated by Gummow *et al*.[13] Consequently, researchers are interested in the analysis of trace metal levels in foods of animal origin.[14–16] The accurate quantification of concentrations of trace metals in tissues and organs of animals may help to elucidate the role and function of trace metals in the living animal and serve as biological indicators of the status of pollution by these metals in the environment.[5,6] Thus, it is necessary to establish effective tests for accurate monitoring of heavy metal residues in meat.

Since meat is a significant part of the South African diet, its heavy metal content may contribute significantly to the heavy metal intake of the consumer. Thus, levels of heavy metals should be determined routinely in meat intended for human consumption. Various spectrometric techniques such as flame-atomic absorption spectrometry (F-AAS),[17,18] electrothermal-atomic absorption spectrometry (ET-AAS),[6] inductively coupled plasma-optical emission spectrometry (ICP-OES)[3,5] and inductively coupled plasma-mass spectrometry (ICP-MS)[10,19] have been used.

The ICP-MS is becoming the instrument of choice for monitoring the levels of toxic metals in food.[15,19] Compared to ET-AAS or ICP-OES, this technique has some clear advantages, including simultaneous measurement capability, coupled with very low detection limits.[19] Furthermore, it offers a wider linear dynamic range that enables the determination of major and trace elements at the same sample injection.[20] Additionally, the ICP-MS provides simpler spectral interpretation and isotopic information, in comparison with the ICP-OES.[21]

Spectral and non-spectral interferences can vary, depending on the food matrix being analyzed.[15] Spectral interferences such as $^{40}Ar^{12}C^+$, $^{1}H^{35}Cl^{16}O^+$, $^{40}Ar^{13}C^+$ and $^{37}Cl^{16}O^+$ on ^{52}Cr or ^{53}Cr must be corrected or reduced to manageable levels when using ICP-MS analysis. The dynamic reaction cell (DRC) and/or collision cell technology (CCT) has proved to be an effective method for alleviating such spectral interferences in ICP-MS analyses.[22]

*To whom correspondence should be ddressed. E-mail: mccrindleRI@tut.ac.za

In previous work where a DRC-ICP-MS was used, the reaction gas O_2 was found to be more efficient than CH_4 in breaking such molecular ions.[23] Thus, the DRC-ICP-MS method with O_2 as reactive gas, was used to reduce polyatomic interferences during quantification of Cr.

The objectives of the study were: (a) to determine the levels of V, Cr, Mn, Sr, Cd, Pb and U in bovine organ samples from possibly polluted areas of Tlokwe (North West, South Africa) using DRC-ICP-MS, and (b) to assess how these metals are distributed in different tissues and organs of bovine origin.

2. Experimental

2.1. Reagents and Standards

All solutions were prepared using ultra-pure reagents. The water used in this work was doubly deionized with the final stage of deionization provided by a Milli-Q water purification system (Simplicity UV, France). High purity HNO_3 (65 %, Suprapur, Merck, Darmstadt, Germany) was used for cleaning glassware and digesting meat, liver, kidney, fat and bone samples throughout this work.

A stock standard solution containing 1000 mg L^{-1} V, Cr, Mn, Sr, Cd, Pb and U obtained from Spectrascan (TEKNOLAB A/S, Kolbotn, Norway) was used for preparing calibration standards. The calibration solutions were prepared from the stock solution using deionized water (18.2 MΩ cm) immediately before analysis. The mass calibration stock solution containing Ba, Be, Ce, Co, In, mg, Pb, Rh and U at 10 μg element L^{-1} was obtained from PerkinElmer (Concord, Ontario, Canada). Instrument grade argon, oxygen and methane gases (Afrox, South Africa) were used for DRC-ICP-MS.

Standard solutions were prepared daily by appropriate dilutions of stock standard 1000 mg L^{-1} of each element (TEKNOLAB A/S, Kolbotn, Norway). Quantification of trace element concentrations were performed, establishing calibration curves with external standards prepared in 1 % v/v ultrapure HNO_3 for analysis of samples. The calibration curve was made from five points and the blank. The 10 mg L^{-1} internal standard solution was prepared from a single element standard solution (1000 mg L^{-1}) of Ga, In, Tl and Th supplied by Spectrascan (TEKNOLAB A/S, Kolbotn, Norway).

A bovine muscle reference material (NIST-RM 8414, Gaithersburg, MD, USA) and a bovine liver reference material (NIST-RM 1577b, Gaithersburg, MD, USA) were used to check the accuracy and precision of the method employed to determine trace elements in muscle, liver, kidney, fat and bone samples.

2.2. Apparatus

A LP3 model freeze-dryer (Jouan, France) was used to dry muscle, liver, kidney and fat samples. A stainless steel blender obtained from Boardmans (Cape Town, South Africa) was used to homogenize the freeze-dried meat, liver, kidney and fat samples. A drill (RYOBI, MODEL HBD6E 5SPEED, Bench Drill Press, South Africa) was used to obtain a fine powder from bone samples. The MARS 5 microwave digestion system (CEM Corporation, USA) was employed for the mineralization of freeze-dried meat, liver, kidney, fat and bone samples. Teflon XP-1500 Plus vessels, allowing a maximum decomposition pressure of 800 psi and temperature of 240 °C, were used for digestion of all samples. The High Pressure Digestion Vessel Accessory Sets (CEM Corporation, USA) permits simultaneous processing of up to 12 XP-1500 Plus vessels. At full power, the MARS delivers approximately 1200 W of microwave energy at a magnetron frequency of 2450 MHz.

All glassware was washed with detergent and water. After being rinsed with deionized water (18.2 MΩ cm) three times, it was soaked in 10 % HNO_3 (v/v) for 24 h. This solution was discarded and the glassware was soaked again in 10 % HNO_3 (v/v) for 24 h. The glassware was then rinsed three times with deionized water with a resistivity of 18.2 MΩ cm, and dried before use.

2.3. Instrumentation

ICP-MS measurements were performed by a quadrupole ELAN DRC-e spectrometer (PerkinElmer SCIEX, Concord, Ontario, Canada), equipped with a DRC. The sample delivery system consisted of a PerkinElmer Auto Sampler Model AS-93 Plus with as93f.try tray, peristaltic pump and a cross-flow nebulizer with a Scott-type double-pass spray chamber. The ICP-MS in standard mode was used during quantification of V, Mn, Sr, Cd, Pb and U. The DRC mode was employed during determination of Cr in bovine meat samples. DRC conditions were selected to give the best compromise conditions. Details of the instrumentation and the operating conditions are summarized in Table 1.

Table 1 Instrumental operating conditions of PerkinElmer ELAN DRC-e ICP-MS.

Operating parameter	Setting
Plasma power output	1300 W
RF generator frequency	40 MHz
Analog stage voltage (volts)	–1850
Pulse stage voltage (volts)	1050
Main water temperature (°C)	19
Interface water temperature (°C)	31
Torch box temperature (°C)	32
Lens voltage (volts)	7
Argon flow rate (L min^{-1})	Plasma: 15, auxiliary: 1.2, nebulizer: 0.82–9.5
DRC gas	O_2
DRC gas flow rate (mL min^{-1})	0.85
DRC rejection parameter q (Rpq)	0.5
DRC rejection parameter a (Rpa)	0
Nebulizer type	Cross-flow
Spray chamber type	Ryton®, double-pass
Interface	Pt sampler and skimmer cones, i.d. 1.1 and 0.9 mm, respectively
Torch	Standard quartz torch
Data acquisition	Peak hopping; dwell time per AMU 40 ms, sweeps/reading 60, number of replicates 3

2.4. Sampling and Sample Preparation

Muscle, liver, kidney, fat and bone samples were collected from cattle carcasses from an abattoir in Tlokwe (North West Province, South Africa) situated near a mining area. A slice of muscle of approximately 200 g was taken with a clean stainless steel knife from each carcase and placed in a plastic bag. Organ samples and bone were taken from offal. All samples were placed in a cooler box at 4 °C and transported to the laboratory on the same day. On arrival at the laboratory the samples were kept in a deep freeze at –22 °C until taken out for analysis. Prior to analysis the samples were thawed, cut into small pieces using a stainless steel knife and 50 g of each sample was transferred into a 250 mL conical flask and kept in a freeze-drier overnight. The samples were then freeze-dried for 24 h. Freeze-dried samples were homogenized using a blender (500 W, stainless steel bottom, 1.5 L glass jug, BBG52). The blender was cleaned well between samples, first with diluted detergent followed by tap

water and then deionized water. The powdered samples were stored in air sealed cartel round bottles. The bone samples were dried in an oven at 60 °C. A drill was employed to obtain a fine powder from bone samples.

About 0.300 g of homogenized freeze-dried meat samples or bone powder samples were transferred into each of XP-1500 Plus microwave digestion vessels. To each vessel, 1 mL of deionized water and 3 mL of 65 % HNO_3 were added. The vessels were then sealed and placed in the MARS 5 microwave digestion system. The samples were mineralized using the following programme: pressure control, 10 min ramp, 10 min hold, maximum pressure 350 psi and maximum temperature 210 °C. The resulting clear solution of the digested sample was quantitatively transferred into 25 mL volumetric flasks. A 25 μL portion of 10 mg L^{-1} internal standards (Ga, In, Tl and Th) was added to each flask, and the flasks were filled up to the mark with deionized water. A blank solution and reference materials were also treated the same way as the samples prior to analysis. All solutions were prepared in triplicate.

2.5. Sample Analysis

Trace element concentrations in bovine meat samples were determined using the ELAN DRC-e ICP-MSinstrument, equipped with a cross-flow nebulizer, platinum cones and a peristaltic sample delivery tube. The instrument underwent 45–60 min routine conditioning and optimization procedures prior to each measurement series. The operating conditions for ICP-MS measurements were optimized daily by monitoring signals produced by a multi-elemental solution containing 10 μg L^{-1} Ba, Be, Ce, Co, In, mg, Pb, Rh and U in the graphics mode of analysis. Those conditions, which maximized ^{115}In, ^{24}Mg and ^{208}Pb signals, were selected.

Concentrations of trace elements in all samples were determined using an external calibration curve. Blank, standard and sample solutions were nebulized and each solution of standard or sample was followed by introduction of deionized water for at least 1 min, to rinse the sampling system. This was done to avoid contamination of other solutions. Three independent replicates of each sample were analyzed, and the concentrations were calculated using the average of each value. The blank samples were also analyzed, and the intensity of each analyte in the blank sample was subtracted from that of the sample.

2.6. Determination of Cr in Meat Samples

Concentrations of total Cr in meat samples were determined using DRC-ICP-MS, applying the method reported previously.[23]

2.7. Selection of Internal Standards

In this study, ^{69}Ga, ^{115}In, ^{205}Tl and ^{232}Th were used as internal standards for V, Cr and Mn; Sr and Cd; Pb; and U, respectively. All digested samples, blanks and calibration standards were spiked with ^{69}Ga, ^{115}In, ^{205}Tl and ^{232}Th internal standard solution, to obtain a final concentration of 10 μg L^{-1}. Using internal standards allowed accurate and precise quantification of trace elements in meat samples and reference materials. Thus, external calibration with ^{69}Ga, ^{115}In, ^{205}Tl and ^{232}Th as internal standards was employed for final quantification of trace elements in meat samples.

2.8. Determination of Limits of Detection (LOD)

To determine the LOD of the whole analytical procedure, reagent blanks were prepared following the same procedures for the quantification of trace elements in the samples. The intensities of 10 blanks were measured. Standard deviations were calculated from the intensity readings of these 10 blanks. The LODs for the species under study, based on three times the standard deviation (3σ) of the average of 10 individually prepared blank solutions, were calculated.

2.9. Quality Assurance/Quality Control Performance

For the assessment of the accuracy of the concentration of trace elements quantified in meat samples, a bovine muscle reference material (NIST-RM 8414, Gaithersburg, MD, USA) and a bovine liver reference material (NIST-RM 1577b, Gaithersburg, MD, USA) were used.

2.10. Statistical Analysis

The observed and certified values of reference materials were compared through *t*-test ananlysis at 5 % level of significance.

3. Results and Discussion

3.1. Limit of Detection (LOD)

Reagent blanks were prepared following the same procedure for the mineralization of bovine liver, kidney, muscle, fat and bone samples. The intensities of 10 blanks were measured under optimum conditions. Standard deviations were calculated from the intensity readings of these 10 blanks. Detection limits were determined for V, Cr, Mn, Sr, Cd, Pb and U. The LODs, based on three times the standard deviation of the average of 10 individually prepared blank solutions, are shown in Table 2.

Nardi *et al*.[19] reported detection limits of 5.0, 10, 5.0, 12, 0.2 and 4.0 ng g^{-1} for V, Cr, Mn, Sr, Cd and Pb, respectively, in 18 different types of food samples, including meat, using ICP-MS. These values are 75.6, 156, 45.4, 179, 28.6 and 9.3 times higher than detection limits obtained using our method for V, Cr, Mn, Sr, Cd and Pb, respectively. Thus, our method provided better detection limits for the elements analyzed.

Table 2 Limit of detection (μg L^{-1}) of the procedure employed for determination of trace elements in bovine liver, kidney, muscle, fat, and bone samples.

Element	LOD
V	0.066
Cr	0.064
Mn	0.11
Sr	0.067
Cd	0.007
Pb	0.43
U	0.006

3.2. Validation of the Method

A bovine muscle powder reference material (RM 8414) and a bovine liver standard reference material (SRM 1577b) were analyzed to test the accuracy of the method. The reference materials were treated exactly the same way as meat samples. The results obtained for the analysis of the certified reference materials by DRC-ICP-MS are presented in Table 3, along with certified values. A paired *t*-test was done to check if the certified and observed values were significantly different. The results were in good agreement with NIST certified values at a 95 % confidence level, demonstrating the accuracy of the method for analysis of liver, kidney, muscle, fat and bone samples. The certified values for Cr in SRM 1577b and for U in both reference materials, were not available.

Table 3 Analysis of bovine muscle reference material (RM 8414) and bovine liver standard reference material (SRM 1577b)

Element	Concentration in mg kg^{-1} of elements in RM 8414		Concentration in mg kg^{-1} of elements in SRM 1577b	
	Observed	Certified	Observed	Certified
V	0.0052 ± 0.001	0.005	0.115 ± 0.004	0.123
Cr	0.073 ± 0.013	0.071 ± 0.038	na[a]	na
Mn	0.34 ± 0.030	0.37 ± 0.09	9.45 ± 0.960	10.5 ± 1.70
Sr	0.056 ± 0.005	0.052 ± 0.015	0.140 ± 0.01	0.136 ± 0.001
Cd	0.014 ± 0.002	0.013 ± 0.011	0.44 ± 0.04	0.50 ± 0.03
Pb	0.40 ± 0.05	0.38 ± 0.24	0.132 ± 0.007	0.129 ± 0.004
U	na	na	na	na

[a]na – not available.

3.3. Selection of DRC-ICP-MS Conditions for Cr

The effect of DRC operating conditions on the alleviation of polyatomic interferences at the masses of Cr was reported previously.[23] The reaction gas used for the DRC system was O_2. In this experiment, the rejection parameter q (Rpq) value of 0.5 and an O_2 flow rate of 0.85 mL min^{-1} were used.

3.4. Total Concentration of Trace Elements in Bovine Liver, Kidney, Muscle, Fat and Bone Samples

Total trace element concentrations in bovine liver, kidney, muscle, fat and bone samples were measured using DRC-ICP-MS. Table 4 shows the total concentration of trace elements (expressed in dry weight) for the samples analyzed.

The concentrations of trace elements in bovine liver, kidney, muscle, fat and bone samples differed for each metal. The highest concentrations of Sr (142 μg g^{-1}), Cd(1.35 μg g^{-1}), Pb (0.62 μg g^{-1}) and U (0.009 μg g^{-1}) were detected in bone samples. A Sr level as high as 142 μg g^{-1} was recorded in bovine bone, probably due to the affinity of this tissue for Sr.[2] Moreover, Sr can substitute Ca in many physiological processes.[24]

Gummow *et al*.[25] detected V concentrations of 0.7 mg kg^{-1} in the liver and 1.15 mg kg^{-1} in the kidney of cattle (mainly calves) that had shown signs or died of chronic V poisoning. Gummow *et al*.[13] later assessed the concentration of the same element in tissues of South African cattle with high background concentrations of V. These authors found maximum concentrations of 11.51 and 5.37 mg kg^{-1} in the liver and kidney, respectively. In this study, the highest concentrations of V in the liver and kidney of bovine were found to be 0.04 and 0.14 mg kg^{-1}, respectively. However, V is rapidly excreted and does not bioaccumulate so only recent or ongoing uptake can be measured in kidney samples. These results are much lower than the reported values for the affected cattle. V is present at trace levels in a variety of commonly consumed foods, including meat. The daily dietary intake in humans has been estimated to vary from 10 μg to 2 mg of elemental V, depending on the environmental sources of this mineral in the air, water and food of the particular region tested.[26]

Kramer *et al*.[27] determined Cr levels as high as 166, 162 and 166 mg kg^{-1} in bovine liver, kidney and muscle, respectively. These values were much higher than those obtained in this study. The concentrations of Cr in organs of bovine were within the same range with our findings from cows' milk.[14,23] Abou-Arab[17] reported the highest concentration of Mn in bovine liver, which was in good agreement with our finding. The other organs contained far lower concentrations of Mn compared with the liver.

Cd and Pb were mainly accumulated in bovine liver, kidney and bone samples as would have been expected from the literature.[7,28] The highest concentrations of these metals were observed in bone samples. It is generally believed that high skeletal Cd and Pb levels are characteristic of chronic exposure to these toxic metals.[28] The bovine kidney contained higher concentrations of Cd and Pb than the liver. The same trend has been reported in the literature.[7] The results are also in agreement with previous studies in sheep and horses,[11] which indicate that these tissues are the main organs for Cd and Pb accumulation. Cd accumulates in the kidney and the liver because its rate of elimination from these organs is relatively low.[18] This is partly due to binding of Cd to sulfhydryl groups in the protein metallothione in in the kidney and liver.[17]

Sedki *et al*.[6] quantified higher concentrations of Cd in kidneys and livers of bovines than in muscle. The highest concentrations of Cd detected in kidneys and livers of these samples were 10.3 and 5.1 μg g^{-1}, respectively. Nriagu *et al*.[7] detected levels of Cd and Pb in bovine kidneys and livers in Jamaica. They reported average concentrations of 33.1 and 10.1 μg g^{-1} Cd in bovine kidney and liver samples, respectively. The reported[7] average concentration of Pb in bovine kidneys and livers were 0.523 and 0.162 μg g^{-1}, respectively. In our study, the highest concentration of Cd observed in bovine kidney and liver samples were 0.51 and 0.16 μg g^{-1}, respectively. We also determined 0.23 and 0.11 μg g^{-1} of Pb in bovine kidney and liver samples, respectively. The reported values were much higher than those obtained in our study. Any comparison of our data with those of other studies must be done cautiously because of differences in analytical methodologies employed, physiological conditions of the animals (whether lactating, pregnant or sickly), age classification of the animal population and the nature of the feed.[7]

It was found that U was below detection limit of the method (<0.006) in liver, muscle and fat samples. The concentrations of U in bovine kidney samples varied from 1.42 to 3.31 ng g^{-1} and its concentrations in bone samples ranged from 3.50 to 8.62 ng g^{-1}. Generally, animals accumulate less U in their tissues, although hen's eggs, kidneys and livers can accumulate relatively higher concentrations.[10] Pork, beef, chicken and mutton contain 1.5 to 3.1 μg U kg^{-1}.[10] These results were in the same range as U levels obtained in bovine kidney samples in our study.

The consumption of meat from livestock from the study area (Tlokwe) perhaps contributed significantly to the average daily metal intake by humans. According to Kan and Meijer[29] muscle is not likely to indicate high levels of heavy metals when animals are exposed *via* diet, whereas the kidney on the other hand, often shows an increase in heavy metal concentration after dietary exposure.

At present, there are no recorded maximum residue levels (MRLs) for heavy metals in meat. Codex Alimentarius Commission[30] has established a limit for Pb in meat, offal and fat. Commission of the European Communities[31] has also set MRLs for Cd and Pb in liver, kidney and muscle. The values set by

Table 4 Concentrations of trace elements in bovine liver, kidney, muscle, fat and bone samples obtained from an abattoir in Tlokwe, South Africa.

Sample ID	Concentrations of elements in ng g^{-1} dry weight (mean± SD)						
	V	Cr	Mn	Sr	Cd	Pb	U
Liver 1	31.0 ± 2.7	192 ± 2.8	6800 ± 660	76.7 ± 3.7	62.6 ± 4.6	111 ± 8.9	<0.006
Liver 2	40.2 ± 3.7	131 ± 1.9	6570 ± 290	70.0 ± 6.6	163 ± 14	91.2 ± 1.8	<0.006
Liver 3	36.8 ± 3.7	111 ± 1.6	7030 ± 690	67.1 ± 4.9	39.1 ± 2.1	<0.43	<0.006
Liver 4	24.0 ± 2.3	130 ± 2.1	7230 ± 580	108 ± 4.9	36.9 ± 1.9	<0.43	<0.006
Liver 5	40.8 ± 4.0	150 ± 2.4	6850 ± 490	105 ± 7.6	29.4 ± 1.1	98.5 ± 5.1	<0.006
Liver 6	40.2 ± 3.8	100 ± 1.6	7230 ± 840	64.1 ± 5.5	63.2 ± 5.5	102 ± 2.9	<0.006
Liver 7	28.1 ± 2.5	229 ± 3.8	7630 ±380	88.6 ± 8.5	31.6 ± 2.3	<0.43	<0.006
Liver 8	26.7 ± 2.2	116 ± 2.9	7330 ± 610	80.6 ± 3.7	60.4 ± 1.6	97.4 ± 7.9	<0.006
Kidney 1	83.4 ± 2.7	323 ± 8.5	3040 ± 100	376 ± 28	508 ± 17	122 ± 11	1.89 ± 0.22
Kidney 2	102 ± 7.5	193 ± 2.9	5770 ± 360	238 ± 15	394 ± 30	<0.43	3.31 ± 0.14
Kidney 3	133 ± 13	260 ± 3.2	4290 ± 360	480 ± 17	314 ± 3.6	109 ± 8.5	3.06 ± 0.32
Kidney 4	106 ± 12	121 ± 2.4	4250 ± 250	192 ± 14	234 ± 13	96.2 ± 11	1.89 ± 0.079
Kidney 5	140 ± 14	181 ± 3.5	4670 ± 280	282 ± 3.8	202 ± 12	112 ± 4.1	<0.006
Kidney 6	63.5 ± 4.9	151 ± 2.8	4170 ± 420	185 ± 5.4	364 ± 30	79.4 ± 6.3	1.42 ± 0.068
Kidney 7	76.6 ± 3.3	151 ± 3.6	4630 ± 220	341 ± 8.7	224 ± 11	234 ± 3.5	1.56 ± 0.17
Kidney 8	56.4 ± 1.8	386 ± 10	4360 ± 300	225 ± 18	425 ± 18	89.6 ± 6.1	<0.006
Muscle 1	27.1 ± 0.54	11.4 ± 0.73	806 ± 63	159 ± 13	10.2 ± 0.28	<0.43	<0.006
Muscle 2	21.1 ± 1.5	72.6 ± 2.6	569 ± 32	84.1 ± 1.2	4.78 ± 0.22	<0.43	<0.006
Muscle 3	23.9 ± 2.3	123 ± 1.7	510 ± 40	70.8 ± 1.2	17.3 ± 2.0	<0.43	<0.006
Muscle 4	29.9 ± 0.8	144 ± 2.2	372 ± 16	62.4 ± 4.1	26.1 ± 7.6	<0.43	<0.006
Muscle 5	33.1 ± 1.5	129 ± 1.2	1180 ± 100	56.4 ± 2.8	18.3 ± 1.6	<0.43	<0.006
Muscle 6	32.5 ± 2.0	114 ± 1.3	230 ± 17	86.1 ± 6.9	30.0 ± 2.1	<0.43	<0.006
Muscle 7	16.4 ± 0.48	258 ± 3.4	480 ± 26	65.2 ± 6.5	12.1 ± 1.1	<0.43	<0.006
Muscle 8	27.3 ± 2.2	499 ± 3.9	312 ± 21	73.8 ± 4.4	31.7 ± 2.0	<0.43	<0.006
Fat 1	16.5 ± 1.2	128 ± 1.8	213 ± 15	37.9 ± 1.3	20.2 ± 1.8	<0.43	<0.006
Fat 2	25.9 ± 2.6	111 ± 2.8	597 ± 53	57.2 ± 1.5	21.9 ± 1.0	<0.43	<0.006
Fat 3	21.8 ± 2.0	193 ± 3.8	271 ± 20	22.7 ± 1.3	25.9 ± 1.7	<0.43	<0.006
Fat 4	14.7 ± 1.2	105 ± 1.1	67.7 ± 5.3	26.2 ± 2.3	22.6 ± 1.6	<0.43	<0.006
Fat 5	28.6 ± 0.98	267 ± 2.5	552 ± 41	110 ± 3.4	34.1 ± 2.4	<0.43	<0.006
Fat 6	9.53 ± 0.82	112 ± 2.2	127 ± 16	29.8 ± 2.7	35.4 ± 3.7	<0.43	<0.006
Fat 7	15.4 ± 1.4	190 ± 4.5	179 ± 15	63.8 ± 2.8	13.4 ± 1.1	<0.43	<0.006
Fat 8	13.7 ± 1.2	155 ± 2.6	165 ± 9.1	57.2 ± 4.8	9.72 ± 1.5	<0.43	<0.006
Bone 1	23.8 ± 1.5	118 ± 1.9	107 ± 9.5	87000 ± 1300	193 ± 6.3	167 ± 3.8	8.21 ± 0.21
Bone 2	55.1 ± 2.8	345 ± 4.8	507 ± 43	104000 ± 480	1350 ± 53	112 ± 1.3	8.62 ± 0.79
Bone 3	35.3 ± 0.83	243 ± 1.9	305 ± 3.7	112000 ± 200	130 ± 6.6	172 ± 2.7	4.50 ± 0.25
Bone 4	16.1 ± 1.3	94.0 ± 1.7	115 ± 11	82000 ± 2100	613 ± 26	181 ± 3.2	5.21 ± 0.38
Bone 5	11.5 ± 0.63	45.5 ± 1.1	166 ± 15	100000 ± 930	113 ± 5.5	64 ± 1.2	5.96 ± 01.54
Bone 6	25.6 ± 0.71	145 ± 2.5	120 ± 12	109000 ± 1300	36.7 ± 1.9	128 ± 2.3	3.50 ± 0.25
Bone 7	20.8 ± 1.2	123 ± 1.4	118 ± 11	123000 ± 2700	63 ± 3.6	619 ± 6.9	7.54 ± 0.54
Bone 8	28.5 ± 1..4	240 ± 2.9	240 ± 18	142000 ± 2200	995 ± 48	69 ± 1.7	4.87 ± 0.38

Codex Alimentarius Commission and Commission of the European Communities are given in Table 5. For proper comparison of the levels of trace elements in liver, kidney, muscle and fat with MRLs, the concentrations in wet mass basis are given (Table 5). Measured mean water contents for bovine liver, kidney, muscle and fat were 70.5, 78.3, 71.9 and 28.4 %, respectively. To convert the concentrations given for dry mass to wet mass basis, the values should be multiplied by 0.295, 0.217, 0.281 and 0.716 for liver, kidney, muscle and fat, respectively.

Cd and Pb levels found in liver, kidney, muscle and fat of bovine are below the MRLs that have been set by Codex Alimentarius Commission and Commission of the European Communities (Table 5). For safety of human consumption, it would be advisable to establish MRLs for other heavy metals in meat.It should also be emphasized that the MRLs alone cannot guarantee low-risk exposures since they do not address eating habits, smoking, environmental sources and nutritional factors.[7] Due to the high accumulation of Cd and its long half-life, it may

Table 5 MRLs (μg g^{-1} wet mass) for Cd and Pb in liver, kidney, muscle and fat.

Element in								References
Liver		Kidney		Muscle		Fat		
Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	
–	0.1–0.5	–	0.1–0.5	–	0.1–0.5	–	0.1	Codex Alimentarius Commission[30]
0.5	0.5	1.0	0.5	0.05	0.1	–	–	Commission of the European Communities[31]
0.05	0.03	0.11	0.05	0.008	nd[a]	0.03	nd	Maximum levels in this study

[a]nd – not detected.

induce diseases in humans, including osteoporosis and decrease of heart contraction ability, especially in smokers consuming higher levels of Cd in bovine meat products.[32] Cigarette smoke is one of the main sources of Cd that may be dangerous to humans. Cd may also cause disorders by inducing secondary hyperthyroidism, showing an antagonistic effect on the accumulation of Cu, Zn, Se and other elements, disturbing reproductive processes and inducing neoplastic diseases.[32]

This study showed that many of the metals accumulate in bones and kidneys, since they contain high levels of several metals, except for Mn, which is higher in the liver. Sr, Cd, Pb and U are high in bone. Cr and V tend to accumulate in the kidney. This unequal distribution of metals amongst organs is related to differences in the specific physiological functions of these elements and depends on their relative abundance in intracellular ligands able to bind metals, such as metalloproteins.[6]

4. Conclusions

Heavy metals in general may play a role in normal physiological processes but can accumulate to toxic levels, particularly in certain organs. In cattle, which produce meat for human consumption, the relative levels of certain metals in different organs can be very important for several reasons. The first is that animals grazing in areas of South Africa may take in metals, yet the residues are not at dangerous levels but they bioaccumulate over time. The method developed for analysis of bovine organs for heavy metals has the advantage of having better detection levels than previous methods and is more cost effective as several elements can be analyzed simultaneously. It thus becomes possible to differentiate physiologically 'safe' levels and be able to predict the speed at which metals can bioaccumulate in tissues more accurately. The work done was able to substantiate the general perception that certain heavy metals such as Cd and Pb are significantly more likely to be found in the kidney and liver even at the lower detection levels than in meat (muscle tissue). Thus, the meat of animals grazing in areas polluted with heavy metals can be safe for human consumption provided liver, kidney and bones are discarded, as the toxic metals appear to bioaccumulate in these tissues.

The method also has value for routine monitoring of heavy metal residues in meat, bone and organs of cattle, particularly for products designed for export. This technique can determine the heavy metal residues, unlike other techniques currently used in South Africa.

The successful extension of the DRC technique using O_2 reaction gas for determination of Cr in meat samples, revealed its potential for future applications. The method could also be adopted for determination of Cr in other biological matrices.

Acknowledgements

Tshwane University of Technology (TUT) and National Research Fund (NRF) are gratefully acknowledged for financial support.

References

1 S.M. Sabir, S.W. Khan and I. Hayat, *Pak. J. Nutr.*, 2003, **2**, 98–101.
2 E.J. Underwood, *Trace Elements in Human and Animal Nutrition,* Academic Press, New York, 1977, p. 545 ff.
3 D. Demirezen and K. Uruç, *Meat Sci*, 2006, **74**, 255–260.
4 M.L. Alonso, J.L. Benedito, M. Miranda, C. Castillo, J. Hernandez and R.F. Shore, *Arch. Environ. Contam. Toxicol.*, 2002, **43**, 103–108.
5 N.S. Mokgalaka, T. Wondimu and R.I. McCrindle, *Bull. Chem. Soc. Ethiop.*, 2008, **22**, 1–9.
6 A. Sedki, N. Lekouch, S. Gamon and A. Pineau, *Sci. Total Environ.*, 2003, **317**, 201–205.
7 J. Nriagu, M. Boughanen, A. Linder, A. Howe, C. Grant, R. Rattray, M. Vutchkov and G. Lalor, *Ecotoxicol. Environ. Saf.*, 2009, **72**, 564–571.
8 F.G. Bell, S.E.T. Bullock, T.F.J. Hälbich and P. Lindsay, *Int. J. Coal Geol.*, 2001, **45**, 195–216.
9 J.O. Nriagu and J.M. Pacyna, *Nature*, 1988, **333**, 134–139.
10 M. Anke, O. Seeber, R. Muller, U. Schäfer and J. Zerull, *Chem. Erde Geochem.*, 2009, **69**, 75–90.
11 Z.P. Liu, *Sci. Total Environ.*, 2003, **309**, 117–126.
12 C.M. McCrindle, E. Mokantla and N. Duncan, *J. Environ. Monit.*, 2001, **3**, 580–582.
13 B. Gummow, C.J. Botha, J.P.T.M. Noordhuizen and J.A.P. Heesterbeek, *Prev. Vet. Med.*, 2005, **72**, 281–290.
14 A. Ataro, R.I. McCrindle, B.M. Botha, C.M.E. McCrindle and P.P. Ndibewu, *Food Chem.*, 2008, **111**, 243–248.
15 S.G. Capar, W.R. Mindak and J. Cheng, *Anal. Bioanal. Chem.*, 2007, **389**, 159–169.
16 A.A. Farmer and A.M. Farmer, *Sci. Total Environ.*, 2000, **257**, 53–60.
17 A.A.K. Abou-Arab, *Food Chem. Toxicol.*, 2001, **39**, 593–599.
18 B. Koréneková, M. Skalická and P. Nad, *Vet. Arhiv*, 2002, **72**, 259–267.
19 E.P. Nardi, F.S. Evangelista, L. Tormen, T.D. SaintPierre, A.J. Curtius, S.S.D. Souza and F. Barbosa Jr., *Food Chem.*, 2009, **112**, 727–732.
20 A.A. Ammann, *J. Mass Spectrom.*, 2007, **42**, 419–427.
21 G. Horlick and Y. Shao, Inductively Coupled Plasma-Mass Spectrometry for Elemental Analysis, in *Inductively Coupled Plasmas in Analytical Atomic Spectrometry,* (A. Montaser and D.W. Golightly, eds.), John Wiley & Sons, New Jersey, 1992, pp. 551–611.
22 V. Dufailly, L. Noël and T. Guérin, *Anal. Chim. Acta*, 2006, **565**, 214–221.
23 A.A. Ambushe, R.I. McCrindle and C.M.E. McCrindle, *J. Anal. At. Spectrom.*, 2009, **24**, 502–507.
24 S.P. Nielsen, *Bone*, 2004, **35**, 583–588.
25 B. Gummow, S.S. Bastianello, C.J. Botha, H.J.C. Smith, A.J. Basson and B. Wells, *Onderstepoort J. Vet. Res.*, 1994, **61**, 303–316.
26 V. Badmaev, S. Prakash and M. Majeed, *J. Alterin. Complement. Med.*, 1999, **5**, 273–291.
27 H.L. Kramer, J.W. Steiner and P.J. Valley, *Bull. Environ. Contam. Toxicol.*, 1983, **30**, 588–594.
28 M. Berglund, A. Akesson, P. Bjellerup and M. Vahter, *Toxicol. Lett.*, 2000, **112-113**, 219–225.
29 C.A. Kan and G.A.L. Meijer, *Anim. Feed Sci. Technol.*, 2007, **133**, 84–108.
30 Codex Alimentarius Commission. Joint FAO/WHO food standards programme. Codex Committee on methods of analysis and sampling, twenty-eighth session, Budapest, Hungary, 5–9 March 2007.
31 Commission of the European Communities. Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, L77, 1–13.
32 A. Zasadowski, D. Barski, K. Markiewicz, Z. Zasadowski, A. Spodniewska and A. Terlecka, *Pol. J. Environ. Stud.*, 1999, **8**, 443–446.