



Occurrence of Chemical Elements in Ewe Tissues from Undisturbed and Slightly Disturbed Areas in Slovakia

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Abstract

This study aimed to measure concentrations of selected essential and toxic elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Zn) in animal tissues of sheep originating from the area with a slightly environmental burden (Horehronie region) and undisturbed area (Orava region) in Slovakia. Liver, kidney, mammary gland, and muscle from 22 ewes for two consecutive years (2020 and 2021) were analysed by an inductively-coupled plasma optical emission spectrometry. Various concentrations of monitored elements were obtained after subjecting the results to statistical analysis. Contents of five toxic elements (arsenic, cobalt, nickel, lead, antimony), and chrome and selenium, were below the detection limits. Content of cadmium exceeded the maximum permissible level in kidneys from the slightly disturbed area. Although the content of copper did not exceed the permissible limit, the concentration of Cu was higher in the liver in both areas in 2021 compared to the results from other studies. The concentration of lithium in the kidney, liver, and muscle from the Orava region was significantly ($P < 0.05$) higher than that of the Horehronie region. Concentrations of Mg, Ag, Cd, Mo, and Zn were significantly ($P < 0.05$) higher in samples from region Horehronie than that in the Orava region. Statistically significant differences ($P < 0.05$) were noted between concentrations of K, Li, and Mo in kidneys; Fe, K, and Li in the mammary gland; Cu, Zn in the liver and Mn in muscles of sheep across the reference years. In summary, monitoring the

occurrence of elements found in commonly consumed dietary raw materials contributes to ensuring the quality and safety of food. Most of the tissue samples analysed are relatively safe for regular human consumption. However, the concentrations of Cd in the kidneys from the slightly disturbed area and potentially Cu in the liver from both areas pose a health risk for the consumers.

Keywords: *Animal Tissues; Essential Elements; Toxic Elements; Sheep; Slovakia*

Introduction

The presence of various chemical elements in animals is of interest from both animal and human health perspectives. Levels of these essentials and toxic elements can indicate the nutritional status of animals and the value of the animal products. Meat and meat products are important components of the human diet as a source of high-quality protein and highly bio-available essential trace elements (Biel et al., 2019). Monitoring the levels of contaminants such as residues and heavy metals in living organisms to protect human health and ensure the quality and safety of food is a very important part of research and national programmes (EFSA, 2009). The capacity to concentrate and cumulate different metals for each organ was calculated. The liver and kidneys are organs which remove toxic metals from the body and end up accumulating them, and because of that, they are target tissues for monitoring metal contamination in animals (Swaileh et al., 2009; Akoto et

al., 2014, Abou- Arab, 2001). Cadmium and lead also can accumulate in sheep muscles, though concentrations are naturally lower in comparison with kidneys and livers (Massanyi et al., 2001). Due to grazing of a herd of sheep on contaminated soil, higher concentrations of metals are found in sheep (Sabir et al., 2003) The goal of this study was to investigate and compare the occurrence of essential and toxic elements in sheep's tissues in two different regions of Slovakia with the different environmental load.

Materials and Methods

Collection of samples

Sheep tissues, specifically the liver, kidney, mammary gland and muscle were used as samples in this study. Samples from 22 ewes together were taken for two consecutive years (2020 and 2021) from two areas with different environmental loads located in Slovakia. Farm from Horehronie region represents an area with a slightly environmental burden and farm from Northern Slovakia, Orava region, which is considered as a control group and originates from the potentially undisturbed area. Division of the country according to the degree of pollution is taken from the document Environmental regionalisation of the Slovak republic made by the Ministry of Slovak republic in (Bohuš and Klinda, 2020).

All animals used in this study (5 ewes in 2020 and 6 ewes in 2021 in the Orava region, and the same number of animals in Horehronie) were humanely killed in a registered slaughterhouse. All samples were taken immediately after slaughter and stored in plastic bags in freezers at -18°C until analysis was carried out, same as in the work of Pšenkova and Toman (2020). Used animals were 4-5 years old and they were improved Wallachian x Lacaune breed from region Orava and Tsigai breed from region Horehronie.

Analysis of samples

Concentrations of 22 selected elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Zn) were measured in animal tissues of sheep. A pre-analytical procedure such as homogenization was made at first. The weight of the experimental samples ranged from 1.0 to 2.0 g and was reflected in measurement. The samples were mineralized in the high-performance microwave digestion system Ethos UP (Milestone Srl, Sorisole, BG, Italy) in a solution of 5 ml $\text{HNO}_3 \geq 69.0\%$ (TraceSELECT®, Honeywell Fluka, Morris Plains, USA), 1 ml $\text{H}_2\text{O}_2 \geq 30\%$, for trace analysis (Sigma Aldrich, Saint-Louis, Missouri, USA) and 2 ml of ultrapure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$; 25°C , Synergy UV, Merck Millipore, France). The method of determination consists of heating and cooling phases. Analysis of the elements was determined using an inductively coupled plasma-optical emission spectrometer (ICP OES 720, Agilent Technologies Australia (M) Pty Ltd.) with axial plasma configuration and with auto-sampler SPS-3 (Agilent Technologies, Switzerland). Detections limits ($\mu\text{g}/\text{kg}$) of measured trace elements were follows: Ag 0.3; Al 0.2; As 1.5; Ba 0.03; Ca 0.01; Cd 0.05; Co 0.2; Cr 0.15; Cu 0.3; Fe 0.1; K 0.3; Li 0.06; Mg 0.01; Mn 0.03; Mo 0.5; Na

0.15; Ni 0.3; Pb 0.8; Sb 2.0; Se 2.0; Sr 0.01 and Zn 0.2. and wavelength of determination (nm) follows Ag 328.068; Al 167.019; As 188.980; Ba 455.403; Ca 315.887; Cd 226.502; Co 228.615; Cr 267.716; Cu 324.754; Fe 234.350; K 766.491; Li 670.783; Mg 383.829; Mn 257.610; Mo 204.598; Na 589.592; Ni 231.604; Pb 220.353; Sb 206.834; Se 196.026; Sr 407.771; and Zn 206.200.

Statistical analysis

All results of this study were processed using Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA). All obtained results are listed as mean values with standard deviation. A probability level of $p < 0.05$ and $p < 0.01$ was considered statistically significant.

Results and discussion

Measured concentrations of selected essential and toxic elements in animal tissues are summarized in **Table 1-4**. Concentrations of Li and Mn in the liver were significantly ($P < 0.01$) higher in samples from region Orava (potentially undisturbed area) than in samples from region Horehronie (area with a slightly environmental burden). In liver samples from Horehronie concentrations of Cd, Ag and Mo were significantly ($P < 0.05$) higher than in samples from Orava. Content of K, Mo and Mg in samples of kidneys from Horehronie is significantly ($P < 0.01$) higher in comparison with kidney samples from Orava. However, the concentration of Li ($P < 0.01$) and K ($P < 0.05$) is higher in kidney samples from Orava. Further content of Li is higher ($P < 0.01$) in muscle samples from Orava, on the contrary, lower content in a sample of a mammary gland from the same region was found. Content of Zn was higher ($P < 0.05$) in muscle samples from Horehronie than in samples from Orava. Comparing contents year-on-year, higher ($P < 0.05$) contents of Li in kidney and mammary gland from Orava, K in kidney and mammary gland from Horehronie, Mo in a kidney from Horehronie and Fe in mammary gland from Orava were recorded in 2020. Statistically significantly higher contents were found for K in a kidney from Orava, Fe and Mn in muscle from Horehronie, Li in mammary gland from Horehronie and Cu and Zn in the liver from Horehronie as well.

Content of Ca was considerable higher in samples from all animal tissues from both monitored groups and both years in comparison with study from region Orava and Western part of Slovakia, where it was at level $92.20 \pm 20.32 \text{ mg}/\text{kg}$ and $57.02 \pm 5.17 \text{ mg}/\text{kg}$ for liver, $129.40 \pm 27.88 \text{ mg}/\text{kg}$ and $132.40 \pm 5.31 \text{ mg}/\text{kg}$ for kidney, $196.40 \pm 274.03 \text{ mg}/\text{kg}$ and $63.42 \pm 8.68 \text{ mg}/\text{kg}$ for muscle, $2,224.80 \pm 1,598.11 \text{ mg}/\text{kg}$ and $1,474.00 \pm 1,223.04 \text{ mg}/\text{kg}$ for mammary gland (Pšenková and Toman, 2020). Content of Mg was higher in samples of liver than in the study by Pšenková and Toman (2020) and comparable in samples of kidney and in muscles from both areas. However, while the content of magnesium in the mammary gland in control group was higher, in experimental group it was lower than in control and experimental group in mentioned study. Concentrations of Zn in this study were much lower than in

sheep from Iran (105.19 and 102,05 mg/kg) (Bazargani-Gilani et al., 2016). Although Zn and Cu are essential elements for the human body, their excessive consumption through diet could be toxic and cause adverse health effects, liver and kidney damage or anaemia caused by Cu (ATSDR, 2004) or anaemia and other haematological effects caused by Zn (ATSDR, 2005). Excess of Zn may cause also Cu deficiency (ATSDR, 2005). Chronic Cu toxicosis is found in ruminants but not in monogastric species and only rarely in humans (McDowell, 2003). Ruminants, particularly the sheep, have a higher potential for copper accumulation in their liver than other species and are more susceptible to copper toxicity (Miranda et al., 2006). The highest mean concentration of copper (101.95 mg/kg) was observed in the liver of undisturbed area in 2021, which is comparable with a concentration of Cu in sheep liver from Obuasi, Ghana (106.63±111.24 mg/kg) (Akoto et al., 2014) and lower than highest copper level (126.14 mg/kg) found in the sheep liver from Iran (Bazargani-Gilani et al., 2016). According to Authority ANZF (2001) permissible limit represents 200 ppm. The maximum copper consumption intake for meat and meat products has been proposed as 0.90–30 mg d⁻¹ per person (Alturiqi and Albedair, 2012). However, the concentration of Cu in sheep liver from both regions was significantly higher than 0.040 mg/kg in the organs of Australian sheep (MacLachlan et al, 2016). The highest content of Mn was found in kidneys from areas with a slightly environmental burden and in the liver from the undisturbed area. Manganese is ranked as one of the least toxic trace elements in mammals, mainly because homeostatic mechanisms keep tissue manganese levels within a limited range (Miranda et al., 2006). The mean content of Fe was higher in all our samples in comparison with samples from Saudi Arabia (El-Ghareeb, 2019), similarly in Slovakia (Pšenková and Toman, 2020). The highest mean content of iron was found in kidneys from the Horehronie region in 2021. A concentration of Cd in the liver in group of

animals from Horehronie in 2020 exceeded the MRL (maximum residual limit; 0.5 mg/kg for liver) according to Commission Regulation (EC) No 1881/2006. However, cadmium concentrations under MRL were found in kidney samples from Horehronie, an area with a slightly environmental burden. In an earlier study, concentration of cadmium in liver in an experimental group under MRL was found in Slovakia (Pšenková and Toman, 2020). The high levels of cadmium content in the liver and kidneys are supposedly on account of their specific function- the liver as a storage and metabolic organ and the kidney as an excretory organ (Stoyke et al., 1995; Massanyi et al, 2014). In addition, cadmium appeared only in one sample of muscles from a potentially undisturbed area with a concentration of 1.13 mg/kg which is under MRL but is lower than in a study by Airyae et al (2015), where the mean value represents 2.8±3.7 mg/kg. The mean Cd concentrations in this study in liver and kidney (listed in **tables 1** and **2**) were significantly higher when compared with 0.007 mg/kg and 0.18 mg/kg from Obuasi, Ghana (Akoto et al., 2014) and comparable with 0.33 mg/kg from Lahore (Mariam et al., 2004). Alarming values were shown in the animals raised in the Campania region, Italy in kidney (1.53; 1.22 and 1.1 mg/kg) and in liver (0.72; 0.64 and 0.61 mg/kg) and in muscle (0.16 mg/kg) (Barrasso et al., 2018). The following elements As, Co, Ni, Pb, Sb, and Se were not detected in any animal tissue used in this study at all, Cd was not detected in mammary glands, and Cr was not detected in any samples of liver, kidney, mammary gland and in muscle was detected only in one sample. Mo was not detected in livers and kidneys. Nevertheless, in other countries traces of heavy metals were found: nickel in Iran and Turkey (Ariyae et al., 2015; Tuncer, 2019) lead under permissible limits in Iran (Bazargani-Gilani et al., 2016), Cr in Nigeria (Bristone et al., 2018), As in Saudi Arabia (El-Ghareeb et al., 2019), As and Co in Australia (MacLachlan et al., 2016).

Element	undisturbed area				area with a slightly environmental burden			
	2020 (N=5)		2021 (N=6)		2020(N=5)		2021(N=6)	
	mean	SD	mean	SD	mean	SD	mean	SD
Ag	0.06	0.10	0.02	0.04	0.06	0.09	0.13*	0.07
Al	1.58	2.49	0.82	2.02	0.00	0.00	0.36	0.89
Ba	1.07	1.82	0.27	0.03	0.43	0.29	0.22	0.14
Ca	676.22	996.31	211.56	20.99	218.54	62.48	171.43	83.17
Cd	0.24	0.17	0.23	0.08	0.75	0.74	0.35*	0.08
Cu	67.24	56.85	101.95	81.57	30.99	28.91	93.03	51.09
Fe	70.13	32.31	90.95	46.54	44.39	21.32	57.57	33.77
K	2016.51	612.91	1920.04	53.38	1647.10	288.41	1875.72	64.59
Li	0.01**	0.00	0.01	0.01	0.01	0.00	0.02	0.01
Mg	178.08	38.18	159.62	9.59	142.16	22.12	160.47	13.73
Mn	2.23	1.29	2.71**	0.51	1.49	0.99	1.72	0.31
Mo	1.03	0.71	1.03	0.20	1.13	0.29	1.33*	0.19
Na	751.97	223.16	596.17	50.94	890.23	473.62	579.63	40.00
Sr	2.86	1.15	2.33	0.21	2.15	0.27	1.65	1.26
Zn	35.45	14.39	31.89	2.74	22.80	10.97	35.57	6.18

SD – standard deviation; *P <0.05; **P <0.01

Table 1: Levels of essential and toxic elements in the liver of sheep from undisturbed area and area with a slightly environmental burden (mg/kg).

Element	undisturbed area				area with a slightly environmental burden			
	2020(N=5)		2021 (N=6)		2020(N=5)		2021 (N=6)	
	mean	SD	mean	SD	mean	SD	mean	SD
Ag	x	x	x	x	0.04	0.08	x	x
Al	x	x	0.67	1.64	1.64	3.68	116.35	282.22
Ba	0.47	0.09	0.47	0.16	0.96	1.01	2.25	3.58
Ca	315.00	161.51	293.16	54.40	298.35	157.28	318.93	118.32
Cd	x	x	x	x	1.10	0.77	1.46	0.74
Cu	3.48	0.23	3.61	0.95	30.61	37.19	3.26	0.75
Fe	29.92	4.65	45.00	40.35	47.34	27.24	174.16	366.61
K	1459.02	135.94	1788.94**	170.01	1781.71**	126.01	1597.62	94.60
Li	0.04**	0.02	0.01	0.01	0.01	0.00	0.21	0.44
Mg	130.24	7.19	155.09	20.37	150.31**	8.75	151.10	59.43
Mn	0.61	0.03	0.71	0.31	1.25	0.84	3.57	7.24
Mo	x	x	x	x	0.82**	0.16	x	x
Na	1388.58	207.94	1402.36	501.96	1186.34	556.07	1522.71	112.95
Sr	2.56	1.00	2.47	0.36	2.45	0.54	2.45	2.01
Zn	15.68	1.51	17.76	2.73	x	x	15.70	2.17

SD – standard deviation; *P <0.05; **P <0.01; x – element was not detected

Table 2: Levels of essential and toxic elements in kidneys of sheep from undisturbed area and area with a slightly environmental burden (mg/kg).

Element	undisturbed area				area with a slightly environmental burden			
	2020(N=5)		2021 (N=6)		2020(N=5)		2021 (N=6)	
	mean	SD	mean	SD	mean	SD	mean	SD
Al	x	x	x	x	x	x	1.52	1.68
Ba	0.24	0.03	0.28	0.04	0.23	0.05	0.20	0.15
Ca	221.58	49.52	217.62	15.74	185.27	29.37	222.14	83.67
Cd	1.13	⁽¹⁾	0.19	0.46	x	x	x	x
Cr	x	x	x	x	0.22	⁽¹⁾	0.04	0.09
Cu	1.95	1.13	2.06	0.86	1.59	0.29	1.95	0.35
Fe	11.48	0.83	18.00	7.50	15.37	4.81	21.49	4.14
K	2145.35	137.43	2079.12	299.08	2068.21	109.11	2066.31	149.95
Li	0.01**	0.00	0.01	0.01	0.00	0.00	0.01	0.00
Mg	185.24	13.64	187.54	29.51	191.08	12.10	201.18	15.31
Mn	0.10	0.03	0.22	0.28	0.08	0.02	0.13	0.03
Na	494.71	34.05	x	x	465.85	51.01	412.91	46.93
Sr	2.32	0.45	2.36	0.15	2.07	0.37	1.87	1.39
Zn	15.70	1.95	17.77	1.15	17.59	1.76	19.53*	1.32

SD – standard deviation; *P <0.05; **P <0.01; ⁽¹⁾ only in one sample was selected element detected; x – element was not detected

Table 3: Levels of essential and toxic elements in the muscle of sheep from undisturbed area and area with a slightly environmental burden (mg/kg).

Element	undisturbed area				area with a slightly environmental burden			
	2020 (N=5)		2021 (N=6)		2020 (N=5)		2021 (N=6)	
	mean	SD	mean	SD	mean	SD	mean	SD
Al	1.71	1.58	2.54	2.22	1.31	1.80	10.72	13.96
Ba	8.08	5.69	3.11	2.57	5.92	7.35	7.46	11.36
Ca	3831.52	1957.10	2613.44	2636.74	2566.11	1885.90	2544.16	2505.78
Cu	1.69	0.18	1.59	0.18	1.80	0.19	1.51	0.30
Fe	31.88	6.53	21.78	7.41	28.35	2.82	34.84	20.74

K	1493.28	196.51	1455.43	219.43**	1371.58	159.29	1154.70	68.36
Li	0.02	0.01	0.01	0.00	0.01	0.01	0.03*	0.02
Mg	285.56	129.94	202.19	108.25	225.83	138.53	221.07	164.38
Mn	0.29	0.05	0.33	0.08	0.30	0.02	0.56	0.31
Na	1247.22	277.50	1256.23	267.58	1197.98	269.81	1344.45	171.95
Sr	7.27	3.49	4.35	2.25	6.44	5.15	5.66	5.94
Zn	16.10	7.10	10.90	5.70	11.76	7.55	13.87	8.59
SD – standard deviation; *P <0.05; **P <0.01; x – element was not detected								

Table 4 Levels of essential and toxic elements in the mammary gland of sheep from undisturbed area and area with a slightly environmental burden (mg/kg).

Conclusion

Statistical analysis of the results showed significant differences in concentrations of Cd, Mo, and Zn in animal tissues of the two animal groups. We found as expected that kidneys and liver cumulate more metals than others animal tissues in this study. Consumption of meat and internal organs from Horehronie (area with a slightly environmental burden) are connected to a higher risk of toxicity because of Cd content under maximum residual limit according to Commission Regulation (EC) No 1881/2006 in liver and kidney. In one sample of muscle from Orava region, potentially undisturbed area, Cd content under MRL was found too. Concentrations of Cu in livers from both areas were not exceeded permissible limit, but in 2021 they were relatively higher in comparison with studies from other countries. Regular and prolonged consumption of Cd and Cu in diet, for example via sheep organs with their high concentrations may lead to accumulation of these metals in the human body and cause toxicity. There is a recommendation to reduce consumption of contaminated tissues from animals from these regions, even exclude their consumption for people belonging to risk groups. Continuous monitoring and further research are needed in terms of maintaining food security and safety.

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