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DETERMINATION OF ELEMENTS LEVEL IN SHEEP MILK FROM TWO REGIONS OF SLOVAKIA AND HEALTH RISK ASSESSMENT OF ITS CONSUMPTION

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Abstract

Milk as a nutritional dense component of diet plays a tremendous role in building a healthy society. However, due to environmental pollution, milk can contain heavy metals and trace elements as well which can threaten human health. The objective of this study was to determine and compare the content of 22 elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Zn) in raw sheep milk collected from the area with potentially undisturbed environment (region Stredné Považie) and area with the heavily disturbed environment (region Spiš) in Slovakia. Analysis of the elements was determined using an inductively coupled plasma-optical emission spectrometer with axial plasma configuration and with auto-sampler SPS-3. Concentrations of only 8 elements (Ca, Na, K, Mg, Al, Fe, Li, and Zn) were found above the detection limit in samples from the potentially undisturbed area, while a whole range of elements was found in samples from the area with heavily disturbed region except three elements (Ag, Cd, and Co) which were below the limit of detection. Significantly higher ($P < 0.05$) concentrations of Na, K, and Zn were found in the Spiš region. The highest concentration of four essential elements Ca, K, Mg, Na, and toxic element Al in samples of sheep milk was found. The hazard index (HI) values were higher than 1 in samples from both monitored farms (6.57 from the farm in the Spiš region and 4.95 in the Stredné Považie region). The highest target hazard quotient (THQ) represents the presence of Al (3.95 and 4.92) in both regions and As (2.17) in the Spiš region. THQ of other monitored elements was less than 1, which means these elements would unlikely cause adverse health effects. Our results indicate that exclusive and long-term consumption of milk from spotted farms can potentially lead to adverse non-carcinogenic health effects due to regular intake of heavy metals, especially Al and As, which can be hazardous mainly for risky groups.
Keywords: sheep milk, elements content, risk assessment of consumption, Slovakia

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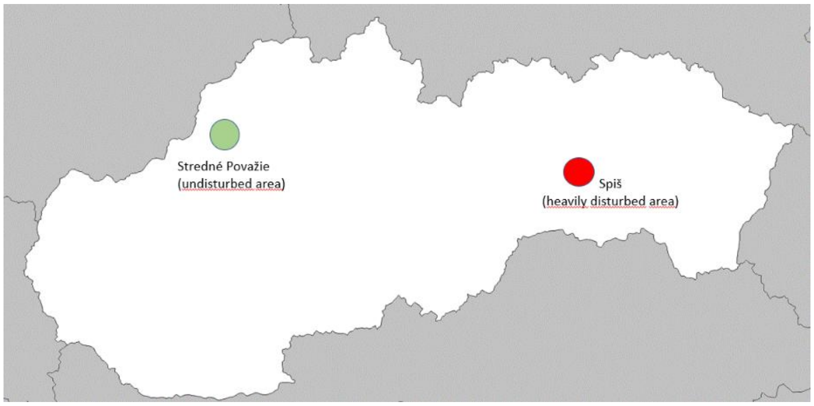
Introduction

Because of rich nutritional profile, good accessibility and preferred taste, milk represents significant role in human nutrition (*Saribal*, 2020; *Pompilio et al*., 2021). Milk and dairy products contain quality proteins, essential fatty acids and micronutrients, mainly Ca, Mg and K (*Licata et al*., 2004; *Kapila et al*., 2013) and its regular consumption has beneficial effect for human health (*Srbely et al*., 2019). The intake of milk dairy products contributes to build bones mass, maintain muscle mass, prevent from civilization diseases (*Aune et al*., 2013; *Fardelonne*, 2019; *Lee at al*., 2018; *Nivine at al*., 2019). Nowadays, the consumption of sheep and goat milk and cheeses is increasing, mainly at the local level, products bought from small farmers (*Kováčová et al*., 2021). However, milk and dairy products may as well contain various amounts of different contaminants, such as residues of pesticides or heavy metals which are widely distributed in the environment. Toxic elements transferred through the food chain may harm human health (*Rahimi*, 2013; *Zhou et al*., 2017). Even small amounts of toxic elements on regular consumption can pose adverse health effect, negatively influence development or function of organs and even cause the outbreak of illness (*Girma et al*., 2014). Intake of toxic elements represents higher risk for vulnerable groups, such children, elderly and people with diseases (*Rahimi*, 2013). Therefore, it is important to monitor the level of trace elements in milk and dairy products which is a major source of nutrition, in childhood in particular. The presence of heavy metals in milk is controlled according to the EU's defined maximum level (EC No 1881/2006). Consequently, the concentration of elements in milk and dairy products indicates their safety and nutritional value (*Zhou et al*., 2017). The aim of the study was to determine the content of essential and toxic elements in samples of sheep milk collected from two farms in Slovakia from areas with different environmental burden, to monitor and compare the current occurrence of elements and to refer to the suitability of the usage for human consumption according to results of risk assessment.

Material and methods

The occurrence of 22 chemical elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Zn) in sheep milk from two selected farms were monitored. The Ministry of the Slovak Republic and the Slovak Environmental Agency has determined the environmental regionalization of Slovakia. The country is divided into three types of environmental quality: regions with a potentially undisturbed environment, and region with a slightly disturbed and heavily disturbed environment. Region Spiš represents heavily disturbed area and region Stredné Považie is considered to be potentially undisturbed area (*Klinda et al*., 2016). Twenty one samples in total were collected from tank milk during period of lactation every two weeks (in 2021 from farm in Stredné Považie and in 2022 from farm in Spiš). Both herds consisted of 450 individuals, ewes from Stredné Považie were Tsigai breed and from Spiš improved valachain x lacaune. Monitored farm from Stredné Považie used conventional way of farming, while farm from region Spiš is an ecological farm.

***Figure 1*: Map of Slovakia with investigated regions location- Stredné Považie and Spiš.**



Analysis of the elements was determined using an inductively coupled plasma-optical emission spectrometer with axial plasma configuration and with auto-sampler SPS-3. Detections limits (µg/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. The legitimacy of the whole method was verified using the certified reference material. The same laboratory method and same instruments were used in a previous study by our research group (*Toman et al*., 2021).

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. Differences in concentrations of the analyzed elements in sheep milk between the farms were compared by the ANOVA and student´s t-test. A probability level of $P < 0.05$ was considered statistically significant.

Concentrations of individual essential elements found in milk were compared with recommended doses for Slovak population (MZ SR, 2015). To health risk assessment of heavy metal present in samples of milk calculation of EDI (estimated daily intake) was made by using following formula:

$$EDI = \frac{(C\ x\ W)}{BW}$$

where C (mg/kg) represents mean level of monitored element, W represents mean daily consumption of milk in Slovakia and BW is mean body weight for man, which is 70 kg. Mean daily consumption of milk in Slovakia is by Statistical Office of Slovakia 0.13 kg per person (Sitárová, 2021). The estimated weekly intake (EWI) of the toxic metals Al and As were calculated by the equation:

$$EWI = EDI\ x\ 7$$

The risk assessment was performed by comparing the calculated EDI and EWI values with set toxicological limits listed in table 2. Consequently, the target hazard quotient (THQ) and hazard index determination (HI) was used for following calculations in the present study. THQ was established by the US Environmental Protection Agency for the estimation of non- carcinogenic risk associated with the reference dose and exposure:

$$THQ = \frac{EDI}{RfDo}$$

where EDI is already explained and RfDo is the reference oral dose (mg/kg/day). The reference dose for Al, As, Ba, Cd, Cr, Li, Mo, Ni, Pb, Sb, Sr, Zn, Cu, and Fe are 0.0004, 0.0003, 0.2, 0.001, 0.003, 0.002, 0.005, 0.02, 0.0035, 0.0004, 0.6, 0.3, 0.04, 0.7 mg/kg BW/day, respectively (US-EPA IRIS, 2018; USEPA, 2011; USEPA, 2012). If the THQ value is greater than 1, potential non-carcinogenic effects could occur, while adverse health effects would be unlikely caused when THQ < 1 (*Boudebbouz et al*., 2022). Since, usually we are not exposed to single contaminant, but to mixtures of them in low doses, hazard index was perfomed to assess the cumulative risk of more than one metal contained in monitored samples by summing the THQ of each metal monitored in the present study. HI value below 1 means that consumption of sheep milk is considered to be safe, whereas hazard index higher than 1 indicates that long term consumption should be considered as a potential cause of adverse health consequences (*Boudebbouz et al*., 2022).

Results and discussion

Various range of monitored elements was found in samples of ewe milk from region Spiš. Concentrations only of three elements, Ag, Cd and Co, were lower than limit of detection, while in ewe milk samples from region Stredné Považie following elements: Mo, Ba, Cr, Cu, Mn, Ni, Pb, Sr, Se, As, Sb, Ag, Cd and Co were not detected. To compare with previous studies in Slovakia concentrations of Cd, Cu, As, Ni and Pb were under limit of detections in samples of ewe milk from undisturbed areas (*Pšenková et al*., 2022; *Tunegová et al*., 2018) and areas with slightly disturbed environment (*Pšenková and Toman*, 2021) and heavily disturbed environment (*Tunegová et al*., 2018). Significant differences were found in levels of Na, K and Zn ($P > 0.05$) and higher concentrations were present in samples from heavily disturbed area. Concentrations of all elements from both areas are listed in *Table 1* below.

Cadmium and lead are considered to be the most widespread xenobiotic metals in the environment and at the same time the most dangerous for human consumption (Kilicaltun et al., 2020). The evidence that even regular and long-term consumption of low amounts of Cd and Pb can pose a health problems, as it is highly cumulative, causes pathological changes and these elements are carcinogenic (Castro-Gonzáles et al., 2019; Amegah et al., 2021). The maximum determined value for lead according to the European Commission (2006) and Codex Alimentarius (2005) is 0.020 mg/L. Mean oncentration of Pb in samples from Spiš region was 0.034 ± 0.042 mg/kg, which is higher than maximum permissible limit, however standard deviation is high in this case too.

Table 1: Comparison of content of elements in sheep from heavily disturbed and potentially undisturbed area (mg/kg)

	Spiš (heavily disturbed area)	Stredné Považie (undisturbed area)
Ca	1669.304 ± 121.094	1704.837 ± 386.896
Na	482.737 ± 62.446*	384.668 ± 52.440
K	1049.076 ± 89.385*	750.640 ± 108.339
Mg	158.446 ± 12.571	150.705 ± 17.874
Mo	0.022 ± 0.018	ND
Al	0.850 ± 0.860	1.059 ±1.309
Ba	0.496 ± 0.041	ND
Cr	0.004 ± 0.008	ND
Cu	0.088 ± 0.010	ND
Fe	2.023 ± 0.456	2.232 ± 0.473
Li	0.012 ± 0.005	0.014 ± 0.003
Mn	0.076 ± 0.030	ND
Ni	0.114 ± 0.029	ND
Pb	0.034 ± 0.042	ND
Sr	1.475 ± 0.211	ND
Zn	4.701 ± 0.419*	2.863 ± 1.619
Se	0.170 ± 0.178	ND
As	0.351 ± 0.141	ND
Sb	0.076 ± 0.086	ND
Ag	ND	ND
Cd	ND	ND
Co	ND	ND

Note: ND = not detected; * $P<0.05$

There are many studies where higher lead content was detected than this limit as well (*Kazi et al*., 2009; *Suturovic et al*., 2014; *Pompilio et al*., 2021). Presence of stronthium in samples from region Spiš is recommended to take into account with sufficient consumption of calcium. Since present level of stronthium in samples represent only 2 % of contribution of total daily intake for stronthium for an adult man, this element is an antagonist of calcium, it adheres to the surface of bones in adulthood and during childhood it can be used to form the mineral part of the bone and thus it is stored in the body for many years. If there is a lack of calcium and protein in a child's diet, strontium can cause poor bone growth. (*ATSDR*, 2020).

In table 2. comparison of EDI (estimated daily intake) of toxic elements from both farm, percentage contribution according to the appropriate set limit for each element and calculated THQ index are listed. *In table 3.* the percentage of contributions of essential elements in monitored farms are listed.

10.17205/SZIE.AWETH.2023.1.007

***Table 2*: Comparison of EDI, % contributions and THQ index for samples from heavily disturbed and potentially undisturbed area**

	EDI (mg/day) Spiš	**EDI (mg/day) Stredné Považie**	**Limits**	**% contribution Spiš**	**% contribution Stredné Považie**	**THQ Spiš**	**THQ Stredné Považie**
Al	0.001579	0.001967	2 mg.kg^{-1} PTWI (JECFA, 2012)	0.08 %	0. 1 %	3.95	4.92
As	0.000652	ND	15 µg.kg^{-1} PTWI (JECFA, 2011)	4.35 %	0 %	2.17	0
Ba	0.000921	ND	0.2 mg.kg^{-1} TDI (WHO, 1990)	0.46 %	0 %	0.005	0
Cr	0.000007	ND	*	*	*	0.002	0
Li	0.000022	0.000026	2 µg.kg^{-1} p-RfD (U.S. EPA, 2008)	1.1 %	1.3 %	0.011	0.013
Mo	0.000041	ND	*	*	*	0.008	0
Ni	0.000212	ND	13 µg.kg^{-1} TDI (EFSA, 2020)	1.6 %	0%	0.01	0
Pb	0.000063	ND	*	*	*	0.02	0
Sb	0.000141	ND	6 µg.kg^{-1} TDI (WHO, 2003)	2.35 %	0 %	0.35	0
Sr	0.002739	ND	0.13 mg.kg^{-1} TDI (WHO, 2010)	2.10 %	0 %	0.005	0
Zn	0.008730	0.005317	**			0.03	0.02
Cu	0.000163	ND	**			0.004	0
Fe	0.003757	0.004145	**			0.005	0.006

ND – element was not detectet in our samples (not possible to calculate EDI, naturally); * tolerable intake was not established or was withdrawm; ** PTDI for risk elements were not calculated in the present study.

Essential elements play an important role in metabolic functions such as maintaining pH homeostasis, osmotic pressure, nerve conduction, muscle contraction (*Bakircioglu et al*., 2016). Inadequate intake of macroelements (Ca, Mg, K, Na), either insufficient or excessive, is associated with serious health problems such as hypertension, osteoporosis, cardiovascular diseases and others (*Bilanžić et al*., 2015). The content of macroelements and microelements in milk can vary significantly. Their amount depends on many factors, such as the age of the animal, health status, lactation period, breeding, season or feed quality, but also the method of milk processing, production process, fermentation or possible fortification. (*Bakircioglu et al*., 2016; *Bilandžić et al*., 2015; *Wang et al*., 2014). Concentrations of calcium in present study were similar with results from previous Slovak studies (*Pšenková and Toman*, 2021; *Pšenková et al*., 2022; *Tunegová et al*., 2016), but lower than in Austria (*Mayer and Fiechter*, 2012) and Croatia (*Antunovic et al*., 2020). Concentrations of Mg in our samples are higher than from previous Slovak studies (*Pšenková and Toman*, 2021; *Pšenková et al*., 2022), but also lower in comparison with samples from Austria (*Mayer and Fiechter*, 2012) and Croatia (*Antunovic et al*., 2020). Levels of Fe were higher in samples from both farms than in Italy (*Miedico et al*., 2016) and Croatia (*Antunovic et al*., 2020).

***Table 3*: The percentage of contributions of essential elements in monitored farms**

	Recommended daily intake	**% contribution Spiš**	**% contribution Stredné Považie**
Ca[a]	1100 mg	19.72 %	20. 09 %
Cu[a]	1400 µg	0.81 %	0 %
Fe[a]	10 mg	2.63 %	2. 90 %
Mg[a]	410 mg	5.02 %	4.77 %
Mn[b]	2.3 mg	0. 43 %	0 %
Se[a]	65 µg	34 %	0 %
Zn[a]	12 mg	5.1 %	3.10 %

[a] (MZ SR, 2015); [b](Institute of medicine, 2001)

To sum up THQs for all monitored elements counted for one scenario (for adults with mean daily consumption) HI for farm in Spiš region (heavily disturbed area) represents value 6.57, while for Stredné Považie region (potentially undisturbed area) HI 4.946 is slightly lower. For the HI values, Al made the largest contribution in both farms, followed by As in samples from Spiš, while THQ for elements Sb, Pb, Zn, Li, Ni, Mo, Fe, Ba, Sr and Cr was lower than 1. In samples from Stredné Považie Al was followed with THQ lower than 1 for Li, Zn, Cu and Fe as well. Other elements were under the limit of detection in those samples. However, we have to mention, that Al in samples from both monitored areas was determined with high variability of results. *Boudebbuz et al*. (2022) found THQ of Ni, Zn, Cu, Fe and Cr < 1 in samples of cows milk from Algeria, Cd higher > 1 for infants, Cr > for children and Pb > 1 for all age groups, except adults with one serving of cow milk per day. The largest contribution for HI was made by Pb, followed by Cr, Cd and Ni. HI values in the study from Algeria were far higher than 1, which means heavy metals may cause adverse effects over a lifetime for all ages with the three counted scenarios (1, 2, and 3 serving cow milk by day) through milk consumption. Hazard quotient in samples of cows milk from Ladakh, a trans-Himalayan high-altitude region, India for Al, As, Ba, Cd, Cu, Fe, Pb, Se and Zn during summer and winter season, both were below the threshold level (< 1). HI for both season were also under the threshold level and As was found to have the highest contribution in HI, while Al the lowest contributon (*Giri at al*, 2021). In Iran, in 36 raw and 36 pasteurized milk samples, THQ for Cd, Cu, Pb and Zn was also lower than 1 (*Sobhanardakani*, 2018).

10.17205/SZIE.AWETH.2023.1.007

Conclusion

Considering that pollutants can be transferred to the human body through food consumption, food safety is great concern to consumers. According to results of sample analysis, we can conclude that there are marked differences between the samples from undisturbed and heavily disturbed area. Since in samples from region Stredné Považie (undisturbed area), the only toxic elements Al and Li were found in low concentrations, in samples from region Spiš (heavily disturbed area) more toxic elements occurred. The most serious concern relates to mean content of Pb, which is higher than maximum permissible level. Significantly higher ($P < 0.05$) concentrations of Na, K and Zn were found in samples from heavily disturbed area. Concentrations of Ag, Cd and Co were under the limit of detection in samples from both monitored areas. The hazard index (HI) values were higher than 1 in samples from both monitored farms (6.57 from the farm in the Spiš region and 4.95 in the Stredné Považie region). The highest target hazard quotient (THQ) represents the presence of Al (3.95 and 4.92) in both regions and As (2.17) in the Spiš region. THQ of other monitored elements was less than 1, which means these elements would unlikely cause adverse health effects. Our results indicate that exclusive and long-term consumption of milk from spotted farms can potentially lead to adverse non-carcinogenic health effects due to regular intake of heavy metals, especially Al and As, which can be hazardous mainly for risky groups.

Considering the results we can assume that the regular long term consumption can be even more dangerous for vulnerable groups, but further calculations are needed.

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