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DETERMINATION OF HEAVY METALS CONCENTRATION IN RAW SHEEP MILK FROM MERCURY POLLUTED AREA

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ABSTRACT

The paper focuses on determining the content of monitored contaminants (Cd, Cu, Hg, Pb and Zn) in 53 samples of raw sheep milk collected in 2013 and 2014 on the sites Poráč and Matejovce nad Hornádom (middle Spiš). The area is characterized by historical mining and metalworking activity (mining and processing of polymetallic ores rich in Hg, Cd and Pb). Currently, the area is one of the most mercury contaminated areas in Central Europe. All statistical analyses were carried out using the statistical software Statistica 10.0 *(*Statsoft, USA). Descriptive data analysis included minimum value, maximum value, arithmetic mean and standard deviation. The results of the studied contaminant content show that the limit value for cadmium (10 µg.kg^{-1}) was exceeded in 25 samples. In the case of lead, the limit value of 20 µg.kg^{-1} was exceeded in 16 cases. The limit value for copper (0.4 mg.kg^{-1}) was exceeded in one case. The limit value for zinc is not defined by a legislative standard. The risk level of the studied contaminants in the samples of raw sheep milk decreases as follows: Cd > Pb > Hg > Cu > Zn. It can be concluded that frequent and long-term consumption of the raw sheep milk originating from the studied sites poses a health risk. The content of the contaminants in the milk and their eventual transition into dairy products should be monitored over a longer term in more detail.

Keywords: former mercury mining area; health hazard; heavy metal; raw sheep milk; Slovakia

INTRODUCTION

Heavy metals and/or trace elements are ubiquitous components of the environment that may be of natural origin: volcanic activity, fires, geogenic origin **(Rutter et al., 2008)**, or anthropogenic origin: metal industry, mining, heavy industry, transportation **(Cui et al., 2005; Navarro et al., 2008; Singh et al., 2005)**. Increasing level of environmental contamination is directly correlated with the level of industrialization **(Tubaro and Hungerford, 2007)**. Metalworking industry and mining of minerals that contain hazardous heavy metals represent a major risk of the environmental contamination, especially of local nature.

Consuming local food poses the greatest risk of intoxication of the consumers by heavy metals that consequently affects their health. **Loutfy et al., (2006)** reported that consumers receive 90% of the total amount of heavy metals by consumption of food from contaminated areas. As a result, human exposure to toxic metals has become a major health risk. Chronic intake of heavy metals above their safe threshold by humans and animals has damaging effects and can cause non-carcinogenic hazards, such as neurologic involvement, headache and liver disease **(John and Andrew, 2011; Lai et al., 2010)**.

Children are particularly sensitive to increased concentrations of heavy metals (especially Hg, Cd and Pb) and arsenic because their tissues and organs accumulate high concentrations of contaminants reflecting in their health during the process of their development and growth. Central nervous system is especially sensitive due to its progressive development and even small amounts of heavy metals can cause irreversible processes resulting in mental retardation and behavioral disorders **(Ataro et al., 2008)**.

Milk and dairy products contain many essential nutrients and their regular consumption is recommended, especialy for young children **(Maas et al., 2011)**. Sheep raw milk has a higher content of essential vitamins and minerals than cow's milk and could be used to cater to consumers' appetite for healthy and safer products **(Bogdanovičová et al., 2015)**. Ovine milk is the most completed natural fluid, one of the most important basic and healthiest raw materials, which plays important role in the dairy nutrition of all population **(Lačanin et al., 2015)**. However, milk and dairy products may contain varying amounts of different toxic contaminants, especially heavy metals **(Ataro et al., 2008)**. In recent years, several reports have indicated the presence of heavy metals in milk and other dairy products **(Kazi et al., 2009; Soylak et al., 2005; Tuzen et al., 2008)**. Due to the fact that milk and milk products are very common food, it is necessary to make great efforts to control the content of the monitored contaminants and at the same time to monitor the quality of individual environmental components that are the main sources of heavy metals in the human food chain **(Caggiano et al., 2005)**.

The paper focuses on the evaluation of the contamination level of raw sheep milk by heavy metals (Cd, Cu, Hg, Pb and Zn). The studied area was formerly characterized by important mining and metal processing activities **(Angelovičová and Fazekašová, 2014)**. Currently, the area is considered as one of the most contaminated sites by mercury, cadmium and lead in Slovakia, but also in Central Europe, which is significantly reflected in the quality of the grown vegetables **(Slávik et al., 2014)**, edible wild mushrooms **(Árvay et al., 2015; Svoboda et al., 2006)**. Thus it is assumed that it will be reflected in the quality and contamination level of the monitored raw sheep milk, the production of which belongs to the major and characteristic agricultural activities in the area.

MATERIAL AND METHODOLOGY

Samples collection

Samples of fresh sheep milk (N = 53) were obtained during 2013 and 2014 from identical individuals in two locations: Poráč (N = 20) and Matejovce nad Hornádom (N = 33). Immediately after the milking, the samples were temporarily stored in PE centrifuge bottles (50 cm^3) and frozen. Just before the analysis, the milk samples were defrosted at room temperature, filtered, homogenized and subsequently analytically processed.

Pre-analytical and analytical procedure

Frozen samples of the sheep milk were defrosted at room temperature just before the analysis. Subsequently, the samples were homogenized by shaking and 2 g were weighed and poured into mineralization tubes. The homogenized sheep milk samples were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., Matthews, NC, USA) in a mixture of 5 cm^3 of HNO_3 (SupraPUR, Merck, Darmstadt, Germany) and 5 cm^3 of deionized water (0.054 $\mu S.cm^{-1}$) from Simplicity185 (Millipore SAS, Molsheim, France). Digestion conditions for the applied microwave system comprised of the heat, which ran up to 150 °C for 10 minutes and was kept at the constant temperature for 10 minutes. A blank sample was carried out in the same way. The sample was subsequently filtered through a quantitative filter paper Filtrak 390 (Munktell & Filtrak GmbH, Bärenstein, Germany) and filled up with deionized water to a volume of 50 cm^3 **(Árvay et al, 2014)**.

The contents of the studied contaminants were determined by flame atomic absorption spectrometry: F-AAS (Cu and Zn) on the SpectrAA 240 FS (Varian Inc., Mulgrave, VIC, Australia), electrothermal atomic absorption spectrometry: GF-AAS (Cd and Pb) with Zeemann background correction on the SpectrAA 240 Z (Varian Inc., Mulgrave, VIC, Australia). Total mercury content (THg) was determined directly in the liquid milk samples (200 µL) by a selective mercury analyzer AMA-254 (Altec, Praque, Czech Republic) based on CV–AAS. Detection limit for F-AAS was 2.0, 0.6 $\mu g.kg^{-1}$ for Cu and Zn, respectively. Detection limit for GF-AAS was 10.0 $ng.kg^{-1}$ for both Cd and Pb. Detection limit for mercury was 1.5 $ng.kg^{-1}$. Certipur® (Merck, Darmstadt, Germany) calibration solution was used for the calibration of all instruments.

Statistical analysis and risks assessment

All statistical analyses were carried out using the statistical software Statistica 10.0 (Statsoft, USA). Descriptive data analysis included minimum value, maximum value, arithmetic mean and standard deviation. The limit of the statistical significance was set up at p <0.05 for all descriptive statistical analyses. To evaluate a health risk resulting from the consumption of raw sheep milk, the obtained data on the content of the studied contaminants were compared with limit values defined by the Codex Alimentarius of the Slovak Republic **(PK SR, 2006)** and EC Regulation 1881/2006 **(EC, 2006)**.

Table 1 Heavy metals in sheep milk samples with descriptive statistics.

Year of collection	Number of samples	Heavy metals in sheep milk samples Median ±SD (range)				
		Hg $\mu g.kg^{-1}$	**Cd** $\mu g.kg^{-1}$	**Pb** $\mu g.kg^{-1}$	**Cu** $mg.kg^{-1}$	**Zn** $mg.kg^{-1}$
				Matejovce nad Hornádom		
2013	19	0.138 ±0.587 (0.079 – 0.286)	1.66 ±1.86 (0.71 – 9.07)	17.3 ±46.5 (ND – 193)	0.14 ±0.06 (0.08 – 0.26)	3.41 ±1.63 (1.02 – 8.04)
2014	14	0.220 ±0.121 (0.063 – 0.450)	8.35 ±9.31 (2.95 – 30.5)	13.6 ±28.3 (ND – 93.6)	0.16 ±0.08 (0.10 – 0.36)	5.51 ±1.07 (4.43 – 7.93)
				Poráč		
2013	10	0.061 ±0.016 (0.036 – 0.079)	12.9 ±4.33 (9.19 – 22.2)	7.85 ±33.9 (6.05 – 113)	0.07 ±0.44 (0.02 – 1.47)	5.53 ±0.99 (3.53 – 6.01)
2014	10	0.068 ±0.025 (0.025 – 0.103)	22.2 ±13.3 (10.1 – 52.9)	12.5 ±9.83 (5.73 – 39.5)	0.12 ±0.14 (0.02 – 0.51)	5.64 ±1.28 (4.10 – 7.97)
Maximum Allowable Levels		**50**[a]	**10**[a]	**1000**[a] **20**[b]	**0.4**[a]	**--**

NOTE: ND – not detected, SD – standard deviation.

[a] Maximum allowable levels of monitored heavy metals - Codex allimentarius of Slovakia **(PKSR, 2006)**.

[b] Maximum allowable levels of monitored heavy metals - Commission regulation (EC) 1881/2006 **(EC, 2006)**.

RESULTS AND DISCUSSION

The contents of the studied heavy metals together with the basic statistical indicators are shown in Table 1. Due to the fact that the sites of interest were characterized in the past by intensive extraction and processing of mercury **(Árvay et al., 2014)**, mercury is considered to be the main heavy metal in terms of quality assessment of the raw sheep milk in this paper. Its content varied in relatively wide intervals within the years, as well as the sites. The highest concentration in terms of the site was recorded in Matejovce nad Hornádom where the mean value of Hg was 0.138 μg.kg^{-1} of the raw sheep milk in 2013 and 0.220 μg.kg^{-1} in 2014. The mean value of the Hg content in the milk from Poráč was about one order of magnitude lower: 0.061 μg.kg^{-1} in 2013 and 0.068 μg.kg^{-1} in 2014. The data are balanced also within the set, as evidenced by the lower standard deviation (Table 1) in comparison with the variability of the Hg values obtained from Matejovce. Such significant differences in the content of the studied contaminant from the sites that are about 2 km apart are due to a significant difference in the atmospheric distribution of emissions from the sources. This is confirmed by other studies **(Angelovičová and Fazekašová, 2014; Svoboda et al., 2000)**. The mercury content in the milk samples from the both sites did not exceed the maximum level of 50 μg.kg^{-1} set by the Codex Alimentarius SR **(PKSR, 2006)**.

The content of cadmium, which is an accompanying element in polymetallic ores mined in the area of interest ranged in a much wider intervals. It is evidenced by the extremely high standard deviations (Table 1). The mean values of Cd content were 9.12 μg.kg^{-1} (2013) and 22.2 μg.kg^{-1} (2014) in the Poráč area and 1.66 μg.kg^{-1} (2013) and 8.35 μg.kg^{-1} (2014) in the Matejovce area. The highest concentration of cadmium was recorded in the Poráč area in 2014 (52.9 μg.kg^{-1}). Large differences in the cadmium content in the sheep milk samples can be caused by several factors such as: seasonality, climatic conditions and variability of feed ration **(Rahimi, 2013)**, since the samples were obtained during outdoor breeding and pasturing. Hygiene standard defined by the Codex Alimentarius sets the contaminant content at 10 μg.kg^{-1}. The obtained results show that the limit value was exceeded in 6 out of 53 samples taken in Matejovce in 2014. In the Poráč, 9 samples exceeded the limit value in 2013 and 10 in 2014. It can be stated that the cadmium content exceeded the limit value in almost 50% of all samples of the raw sheep milk.

The lead content in the milk samples varied at different intervals, depending on the site. The mean Pb content was 7.85 μg.kg^{-1} (2013) and 12.5 μg.kg^{-1} (2014) in the Poráč area. Similarly to the cadmium, the lead content varied widely, which was reflected in the standard deviations (Table 1). In comparison with Matejovce, where the Pb content varied in a higher concentration: 3.17 μg.kg^{-1} (2013) and 13.6 μg.kg^{-1} (2014), the Poráč area seems to be less risky. However, the results show that both sites pose a potential risk resulting from the sheep milk consumption, since the limit value (20 μg.kg^{-1}) set by the EC Regulation 1881/2006 **(EC, 2006)** was exceeded in 9 (2013) and 3 samples (2014) taken from Matejovce and in 2 samples (2013 and 2014) taken from Poráč. Codex Alimentarius SR does not state a maximum allowed content of lead.

Copper and zinc are considered essential micronutrients and their content in food is desirable in an optimal amount **(Maas et al., 2011)**. The copper content in the milk samples varied in a relatively low concentrations compared with zinc. The mean value of the copper content was 0.07 mg.kg^{-1} (2013) and 0.12 mg.kg^{-1} (2014) in the samples from the Poráč site and 0.14 mg.kg^{-1} (2013) and 0.16 mg.kg^{-1} (2014) in the samples from the Matejovce site. Codex Alimentarius SR set the maximum level of copper to 0.40 mg.kg^{-1}. The limit value was not exeeded in any samples on the mean level. However, in the case of individual samples, the limit was exceeded in one sample from the Poráč site taken in 2013 (1.47 mg.kg^{-1}). It can be concluded that in terms of the copper content, consumption of the sheep milk does not pose a health risk. The mean values of the zinc content varied at higher levels. In the samples taken from the Poráč site, the Zn content was 5.53 mg.kg^{-1} (2013) and 5.64 mg.kg^{-1} (2014). The Zn content recorded in the samples taken from the Matejovce site was 3.41 mg.kg^{-1} (2013) and 5.51 mg.kg^{-1} (2014). The content of Zn was relatively balanced as evidenced by the relatively small standard deviations (Table 1). Due to the fact that no legislative standard defines limit values for Zn in milk, it is not possible to make conclusion on the hygienic quality of the sheep milk in terms of zinc content.

CONCLUSION

Evaluation of the contamination level of agricultural products and food ingredients is important in terms of maintaining an adequate health safety of human food chain components, especially in areas that are significantly contaminated by risk elements such as heavy metals. The contents of the studied contaminants in the raw sheep milk samples taken from two areas: Poráč and Matejovce nad Hornádom, ranged in various levels posing different degrees of health risk resulting from consumption of the milk. Mercury was assumed to pose the highest health risk, however, the hygiene standards for this element were not exceeded. The most hazardous contaminant was cadmium. The maximum allowed level of Cd (10 μg.kg^{-1}) was exceeded in 25 out of 53 samples. The limit value of lead (20 μg.kg^{-1}) was exceeded in 16 cases. The copper content exceeded the limit value (0.4 mg.kg^{-1}) in one case. The limit value for zinc is not defined by any legislation. It can be concluded that regular consumption of the sheep milk, in connection with intake of the studied contaminants from other sources, may pose a health risk in the long term. Therefore, it is necessary to monitor the contaminants in the milk, as well as milk products in long-term and more detail.

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