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ANALYSIS OF CONCENTRATIONS OF RISK AND TOXIC ELEMENTS IN SHEEP MILK FROM AREA OF SLOVAKIA WITH POTENTIALLY UNDISTURBED ENVIRONMENT

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ABSTRACT

Concentration of toxic metals in milk, especially in industrial regions, may serve as a direct bioindicator of the quality of milk, which is an important food in human diet. The aim of this study was to find actual contamination of selected area of Slovakia and to determine the effect of lactation stages on the concentration of risk and toxic elements in sheep´s milk of Tsigai breed. Monitoring of environment was performed by sampling of soil, feed, and milk samples from 400 ewes. Selected elements in feed and milk were detected using the AAS-HG, AES-ICP, AAS-ETA, AAS-AMA, and AAS-F and in soil samples by ICP-OES. Concentrations of toxic elements (As, Hg, Pb, Ni) were found in the soil samples, which corresponded to MRL, and their content was not reflected in the samples of feed and milk of sheep and were below the LOQ. The effect of lactation stage was statistically significant only in case of Ca ($P<0.05$), where a significant increase in concentration of this element was found throughout the lactation period. A statistically significant increase of concentrations of Se, Mg, Zn and Fe ($P<0.05$) in sheep milk was recorded only in the late stage of lactation. Pearson´s correlation analysis showed a several very strong and positive correlation relationships in individual lactation stages of sheep. The very low concentrations, below LOQ of selected toxic elements in milk confirmed, that level of contamination of the selected area of Slovakia is very low, and milk from this area of Slovakia is in good hygienic quality and it is safety for direct consumption or for further processing.

Keywords: ewes, contamination, soil, feed, lactation stage

INTRODUCTION

Milk and dairy products represent a simple dietary component and important sources of macro and micronutrients, including essential minerals (**Zhou *et al.*, 2017**). Of the total world milk production of small ruminants, for 36.5 % is sheep milk, and 63.5 %, is goat milk (**FAO, 2017**). In general, all breeds of sheep can be milked, but some breeds are excellent milk producers. Average lactation period of non-dairy breed lactation period of 90-120 days an in dairy sheep, lactation varies from 180 to 240 days. Sheep milk is more popular among consumers in processed form of cultured dairy products like yogurt and cheese (**Mohapatra *et al.*, 2019**). The quality of sheep milk is highly important, especially in paramount importance in controlling the quality of dairy products (butter, cheese) made from it. Fresh sheep milk is rarely consumed compared to sheep dairy product, because of its high fat and total solid contents (**FAO, 2016**).

Sheep´s milk is a high value product in human nutrition, as it contains around 200 active substances (**Horák *et al.*, 2004**), among which it may contain higher amounts of vitamins A, B and E, calcium (Ca), phosphorus (P), potassium (K), and magnesium (Mg) in comparison to cow milk, as analyzed by **Zervas and Tsiplakou (2011)**. Importance of sheep´s milk quality is highlighted also in the research by **Cleays *et al.* (2014)**, which compared quality of human milk with milk of horse, donkey, and sheep´s milk and concluded that sheep´s milk can be suitable and very good alternative to breast milk and infant formula.

Even though milk and dairy products are very good source of bioactive substance, they can contain high concentrations (mg/kg) of toxic trace elements as a result of increased anthropogenic pressure (industrial, agricultural and urban activities) on the environments in which cows, sheep, and goat are raised (**Levkov *et al.*, 2017**). Metals can accumulate up the food chain through biotransformation because they are not biodegradable and this results bioaccumulation and toxicity in environment and humans (**Muhib *et al.*, 2016**). Animals such as cows, sheep, goats, which are source of milk and dairy products assimilate trace metals from food and water, as well as inhaling contaminate atmospheric particulates (**Muhib *et al.*, 2016; Esposito *et al.*, 2017**). Harmful metals such as cadmium (Cd), arsenic (As) and lead (Pb) are elements, which can be transferred from animals to the human diet when people consume animal products from location with contaminated soils, fodder, and/or pastures (**González-Montaña *et al.*, 2019**).

Some toxic elements cause serious health problems even at low concentrations, and it is therefore important to monitor their residual values in animal products. Toxic metals have various effects after penetration into the body, which can cause health problems such as renal failure, nervous system disorders, genetic mutation (**FAO, 2016**), respiratory disorders, neurological disorder, types of cancers, and cardiovascular, infertility and immune system weakening (**Azami *et al.*, 2017**). Lead (Pb) causes damage to the kidneys, central nervous system disorders, anemia, damage of liver, heart and blood vessels, genital systems, problems in digestive tract, and the development of cancer (**Alimardan *et al.*, 2016; Azami *et al.*, 2017; Tavakoli-Hosseinabady *et al.*, 2018**). Cadmium (Cd) accumulates in tissue like the kidneys and liver, causing anemia, and increase in blood pressure. Cadmium is carcinogenic element, especially in the prostate and lungs, where this element causes the tumor develops. The next toxic element - nickel (Ni) can cause local infections and variety of cancers of the blood, brain, and bone (**Ziarati et al., 2016**). The high concentration of Ni in animal or human body, it may occur disruption of the biological activity cells, delayed growth, decreased hematuria and interfering in iron absorption and subsequently poisoning by this element (**Hamidi and Doneshpajooh, 2009**). On the other hand, **Suttle (2010)** state, that milk is richer in essential elements, such as calcium (Ca), zinc (Zn), potassium (K), and phosphorus (P) but milk is not good source of iron (Fe), cooper (Cu), magnesium (Mg). Elements Zn, Cu, and Fe are essential and very important for many biological functions in animal and human body. The absence of these elements contributes significantly to various civilization diseases; however, even high concentrations of these elements can have a negative effect on animal and human health. The level of necessity and toxicity of elements differ from element to element (**Kazi *et al.*, 2009**)

Sheep milk may contain various essential or risk elements which concentrations from a toxicological and nutritional point of view can vary according to internal factors (lactation periods, animal breed, health status) and external factors (environmental conditions, feeding, seasons) (**Llobet *et al.*, 2003**). Small ruminants are animals that are usually fed by a meadow grazing system and therefore these animals are possible considered such as bio-indicators of environment (**Miedico *et al.*, 2016**). In general, monitoring of concentrations of xenobiotics, mainly toxic elements in environment, are considered as a good indicator of contamination in program of soil quality and control and in air quality monitoring (**Caggiano *et al.*, 2005; González-Montaña *et al.*, 2012**).

Therefore, the main goal of this study was to evaluate the state of the environment of the selected area of Slovakia based on the concentrations of essential and selected toxic elements in soil and animal feed and to determine the concentrations of these elements in sheep´s milk due to different stages of lactation.

MATERIAL AND METHODS

Study area

The monitoring of area was provided on farm of Northern Slovakia during the lactation stages of sheep. According to the Environmental regionalization of Slovak Republic (SR) area of Slovakia is divided into three types of environmental quality: 1st environmental quality (regions with potentially undisturbed environment), 2nd environmental quality (convenient environment; regions with a slightly disturbed environment) and the 3rd environmental quality (regions with heavily disturbed environment) (**Bohuš and Klinda, 2020**). To determine the real contamination of the environment and effect of lactation stage on concentrations of selected elements, a sheep farm was selected. This farm is located on the north of SR in the region Orava with potentially undisturbed environment. This area, where the selected farm is located, it may be affected by environmental pressures from industrial production, transport and logistics facilities and waste management facilities. The selection of animals, the method of milking and the living conditions of the animals were the same as we describe in the work **Pšenková *et al*. 2020a**. The farm in monitored area has a herd of sheep of Tsigai breed with total number of animals 400.

Sample collection

The soil samples were collected from places where the animals grazed. Each soil sample was taken at a depth 0-20 cm from different location of pastures and the total number of soil samples was 15 (5 samples of soil in each of lactation stages of animals).
To determine the environmental impact of the selected area, animal feed was also analyzed. Sampling of feed and milk, volume of milk samples, storage of all soil, feed and milk samples was carried out in the same way as in described in our work **Pšenková *et al.* 2020a**. In total, it was obtained 15 feed samples (5 samples from each lactation stages) and 30 samples of milk (10 samples of milk in each lactation stage). Since the farm in monitored area had a herd of 400 ewes, the milk samples were taken from milk tanks immediately after the end of milking. For environmental monitoring of selected area of Slovakia and the occurrence of selected elements in sheep milk, 11 elements were selected: as toxic elements (cadmium, arsenic, mercury, nickel, lead) and risk - essential elements (calcium, selenium, zinc, magnesium, iron, copper).

Sample analysis

All samples were prepared and analyzed in collaboration with a certified testing laboratory Eurofins/ Bel Novamann (Nové Zámky, Slovak Republic). Detailed description of sample preparation and individual steps of sample analysis were performed in the same way as we present in our pervious study **Pšenková *et al.* 2020a**. The concentrations of all selected 11 elements in soil were established by ICP-AES (inductively coupled plasma-atomic emission spectrometry) with Spectrometers Varian 720-ES, Australia, in feed and milk concentrations of Ca, Fe, Mg were analyzed using ICP-AES (inductively coupled plasma-atomic emission spectrometry) with Varian 720-ES, USA. Arsenic and Se in milk and feed were analyzed using HG-AAS (hydride generation atomic absorption spectroscopy) with Spectr AA-220 FS (The Netherlands). Toxic elements Cd, Pb and Ni in milk and feed were analyzed using ETA-AAS (electro thermal atomization atomic absorption spectrometry) with Agilent DUO AA 240Z/240FS, USA) and Hg in milk and feed was analyzed by advanced mercury analyzer and atomic absorption spectrometry (AMA-AAS, Altec CR). Concentrations of Cu and Zn in feed and milk were analyzed using the F-AAS (flame atomic absorption spectrometry) with DUO AA 240Z/240FS, USA.
The quality assurance and preparation of all milk, feed and soil samples were performed in the same process as described in our pervious study **Pšenková *et al.* (2020b)**. For the validation of the analytical methods the limits of detection (LOD) and limits of quantification (LOQ) were evaluated (Table 1).

Table 1 LOD and LOQ limits for risk and toxic elements analyzed

	LOD (mg/kg)			LOQ (mg/kg)		
Elements	**soil**	**feed**	**milk**	**soil**	**feed**	**milk**
Ca	2.3	2.0	2.0	7.0	6.0	6.0
Cu	0.3	0.17	0.017	1.0	0.5	0.05
Fe	3.0	0.17	0.17	10.0	0.5	0.5
Mg	0.3	0.33	0.33	1.0	1.0	1.0
Se	0.06	0.017	0.0067	0.2	0.05	0.03
Zn	2.0	0.17	0.17	6.0	0.5	0.5
As	0.06	0.017	0.01	0.2	0.05	0.03
Cd	0.13	0.0067	0.0013	0.4	0.1	0.004
Hg	0.003	0.0033	0.00067	0.01	0.01	0.002
Ni	0.8	0.033	0.03	2.5	0.1	0.1
Pb	1.0	0.033	1.0	3.0	0.1	0.01

Statistical analysis of data

The all results of this study were processed using a statistical IBM SPSS Statistics 20. The all obtained results are listed as mean values with standard deviation and minimum and maximum and minimum concentrations are also given for all elements. The comparison of average concentrations in individual stages of lactations was analyzed using The Tukey´s HSD test and differences were declared significant at $P<0.05$ level. The Pearsons´s correlation analysis was used to determine the individual relationships between the elements in milk.

RESULTS AND DISCUSSION

The content of toxic and risk elements in milk can be affected by many factors. In addition to the mineral composition of soil and feed and their industrial contamination, these are – species and breed of animals, genetics, lactation stage, age and health status of animals, season, soil origin and environmental factors. Measured concentrations of these elements may also be affected by analytical procedures and cross-contamination during collection and processing. The source of the elements may also be the soil that the animals consume during grazing or are fed with contaminated feed (**Pereira, 2014; Alothman *et al*., 2019**).
Concentrations of soil elements can be influenced by various factors such as fertilizer application, natural processes (rock weathering, leaching of soil elements), soil factors (low soil pH and its changes) or contaminated water intended for irrigation of soil and agricultural crops (**Winkel *et al*., 2015**).
The results of average concentrations of elements in soil and animal feed from selected area of Slovakia are summarized in Table 2 and average concentrations of monitored elements in sheep´s milk are listed in Table 3. When monitoring the environment, through soil analyzes, we found the concentrations of toxic elements, except Cd in the soil, even though it was an uncontaminated area. Concentration of this element was below LOQ. From Table 2 despite the observed average concentrations of toxic elements in the soil, the content of these elements did not appear in animal feed, even though the feed was grown in places of sampled soil. All toxic elements in animal feed were recorded under the LOQ. Analysis of soil samples in this study detected high concentrations of Fe in the soil, however, this high concentration did not affect the Fe concentration in feed and milk. Iron concentrations in feed and milk were significantly lower compared to soil. There was a significant decrease in the concentration of Fe in milk and feed compared to the soil.
The average value of As in soil in monitored area with potentially unpolluted area of Slovakia was 5.70 mg/kg. This concentration corresponded to the value given for unpolluted agricultural land 0.1-40 mg/kg (**López-Alonso, 2012**). This element can enter the soils, for example, through contamination from pesticide application or mining (**Warren *et al*., 2003**), however as recorded **Tóth *et al*. (2016)**, Slovak Republic can be classified as a country with generally lower level of arsenic in the soil. Cadmium was not detected in the soil in monitored area of Slovakia, the measured values were below the LOQ (<0.40 mg/kg). In the literature, soil with a level of 1 mg/kg of Cd is mentioned as contaminated soil. In uncontaminated soils, Cd reaches concentration of 0.1 – 0.2 mg/kg (**Lane *et al*., 2015; Thou *et al*., 2018**). **López-Alonso (2012)** reports Cd concentration in uncontaminated area in the range 0.06-1.1 mg/kg. According to the literature, the soil in monitored area corresponds to the soil with low cadmium values.
The average concentration of Hg in the soil on selected farm was 0.00688 mg/kg. The detected Hg concentration in soil of farm is lower than the concentration 0.5 mg/kg, which is considered normal concentration in soil **López-Alonso (2012).** Nickel was present in the soil on sheep farm in an average concentration 21.60 mg/kg. The total or increased Ni concentrations in soil can be significantly affected by agricultural activity (**Qing et al., 2015**). Average concentration of Ni in this study is similar to concentration which reported **Tahir *et al*. (2017)** during the spring in Pakistan (25.72 mg/kg) and **López-Alonso *et al*. (2012)** in soil taken from an organic farm in Spain (25.4 mg/kg).
The average content of Pb in soil in our work was 27.90 mg/kg. Lead concentrations may also originate in emissions from transport that may have been imported into the area (potentially undisturbed area) or in the use of pesticides in agriculture (**Yu *et al*., 2016**). The average concentrations of Pb which have found correspond with levels up to 20-40 mg/kg, which are considered as a normal as demonstrates this author (**López- Alonso, 2012**).
From the selected risk elements that we observed in our work, was recorded their concentrations in all cases. The most significant result was observed in the concentration of Fe (26620 mg/kg). Iron can be released into the environment during the combustion of coal, biofuels, diesel, and biomass (**Wang *et al*., 2015**). This concentration of Fe is comparable to the concentration of 20999 mg/kg reported by López-Alonso et al. [38] and higher as found by **Kumar *et al.* (2019)**. Concentration of Cu, Se in our samples of soil was very similar as detected **López-Alonso (2017)** (Cu: 26.3 mg/kg; Se: 0.264 mg/kg). **Kumar et al. (2019)** found in agricultural soils significantly higher concentration Cu (48.7 mg/kg) compared to this study. Although concentrations of toxic elements were recorded in soil samples in this study, their concentrations in animal feed were not recorded in compared to our similar study, where we recorded the transfer of toxic elements from the soil to the feed in area with slightly disturbed environment (**Pšenková *et al*., 2020a**).

Table 2 Mean concentrations of risk elements and selected toxic elements in soil and feed of sheep (mg/kg)

Essential elements/Toxic metals	Soil (Mean concentration)	Feedstuff/Green forage (Mean concentration)
Ca	3940.00±23.25	914.00±11.45
Mg	4660.00±2.63	303.00±16.79
Zn	107.00±7.87	7.50±4.5
Se	0.30±0.06	0.081±0.023
Fe	26620.00±235.8	99.00±7.8
Cu	25.90±4.52	2.60±0.13
As	5.70±0.41	<0.030*
Cd	<0.40*	<0.10*
Hg	0.0068±0.012	<0.01*
Pb	27.90±2.47	<0.30*
Ni	21.60±1.58	<0.10*

*Concentrations with this index are below the LOQ (limit quantification)

In samples of milk from monitored area were recorded the highest concentrations of Ca and the lowest concentration in case of Se (Table 3). In cases of all toxic element concentrations and also concentration of Cu were found in milk below the LOQ.

It is evident from Table 4, that in milk of sheep from selected area of Slovakia was found significant increase of concentration of Ca during all stage of lactation ($P<0.05$). An increase of concentration was also observed in case of Se, Mg and Fe between middle and late of lactation stage. In milk from sheep, the concentration of Zn during lactation has changed statistically significant. Between the early and middle stage of lactation, a statistically significant decrease in Zn concentration was recorded, on the other hand, between middle and late of lactation concentration of Zn statistically significant increased ($P<0.05$). Concentrations of all toxic elements in individual stage of lactation were recorded below LOQ.

Concentrations of risk and toxic elements in milk depend mainly on their concentrations in feed. Feed, which is intended for small ruminants or livestock with an increased concentration of contaminants, also causes their higher concentrations in milk (**Iftikhar *et al*., 2014**). This is also confirmed by the authors **Raj *et al*. (2006)**, who reported in their study that pastures used as feed for cattle contained higher levels of Cd and Pb compared to the MRL (maximum residual limit) for animal feed, which let to high concentrations of these elements in milk. In this study, concentrations of toxic elements in feed were below the LOQ in compare to the soil their content was also not found in the milk samples and were also below LOQ. **Antunovič *et al.* (2016)** also found the low concentrations of toxic elements similar to us, but on the other hand compared to the results of the our present study in lactation stages, they found higher concentrations of Cd and Pb (0.011 and 0.035 mg/kg respectively in 2nd lactation day) and higher concentrations of toxic metals in sheep´s milk on the 10th (Cd: 0.004 mg/kg; Pb: 0.022 mg/kg; As: 0.025 mg/kg), 30th (Cd: 0.005 mg/kg, Pb: 0.024 mg/kg; As: 0.028 mg.kg^{-1}), and 60th (Cd: 0.006 mg/kg; Pb: 0.026 mg/kg; As: 0.025 mg/kg) lactation day. In addition to important factors such as the quality of environment, feed, animal breed, the concentration of elements greatly affects the stage of lactation (**Vahčić *et al*., 2010; Zamberlin *et al*., 2012**). The changes in concentrations of toxic elements in sheep milk, which were found in this study also similarly described by the authors **Antunovič *et al*. (2016)**. Similar to previous authors, also **Špánik *et al*. (1997)** state that the lactation period has the most significant effect on the change content of individual milk components. The fat and protein content of milk, which makes up a substantial part of the dry matter, is particularly subject to major changes. Minerals in milk are in the form of salts or are bound in proteins and their content in milk is expressed as a percentage of ashes. In general, during lactation, the content of fat protein, as increases in milk and the content of milk sugar generally decrease with the course of lactation. During the monitoring of the concentrations of essential elements in milk during the lactation stages, we recorded a gradual increase in the concentration of sheep milk in the case of Ca, Mg, which corresponds to the statement of the team of authors **Špánik *et al.* (1997)**, but at the concentrations of Se and Fe they increase only in the 3rd stage of lactation. Similar changes of mineral concentration in milk of ewes in Croatia compared to this study were found by **Antunovič *et al*. (2016)**. Authors reported similarly, as in this study, significant decrease of concentration of Zn between early and middle lactation stage and increase between middle and late lactation stage. In concentration of Ca they found the same statistically significant increase concentration of Ca during the lactation stages compared to the present study. Increased concentration of Ca in milk was found in also of Yanakas sheep in Nigeria during the lactation (0.5125-1.78 g/kg) **(Mwaura and Akinsoyinu, 2010**). In milk of sheep from Bulgaria compared to this study were found higher concentration of Fe (0.8-1.3 mg/kg) and similar concentration of Zn (3.81-4.41 mg/kg) and higher concentration of Se (25.5-17.5 mg/kg) (**Ivanova *et al*., 2011**).

Table 3 Mean concentrations of risk elements and selected toxic elements in sheep´s milk (mg/kg)

Risk elements/Toxic metals	Mean ± SD	Min.	Max.
Ca	1620.00±1.58	1410.00	1890.00
Se	0.04±0.002	0.031	0.063
Zn	4.10±0.16	3.40	4.00
Mg	125.33±1.40	107.00	162.00
Fe	0.66±0.02	0.59	0.81
Cu	<0.50*	-	-
Cd	<0.0040*	-	-
As	<0.030*	-	-
Hg	<0.002*	-	-
Ni	<0.10*	-	-
Pb	<0.10*	-	-

*Concentrations with this index are below the LOQ (limit quantification); SD – standard deviation; min – minimum value; max- maximal value

Table 4 Concentrations of risk elements in sheep´s milk during different stages of lactation (mg/kg)

Elements (essential and toxic)	**Lactation stages (Mean ± SD)**		
	Early	**Middle**	**Late**
Ca	1410.00±1.58^{c}	1560.00±1.40^{b}	1890.00±1.20^{a}
Se	0.031±0.002^{b}	0.031±0,002^{b}	0.063±0.002^{a}
Zn	4.90±0.16^{a}	3.40±0.16^{c}	4.00±1.58^{b}
Mg	107.00±1.58^{b}	107.00±1.58^{b}	162.00±1.60^{a}
Fe	0.59±0.02^{b}	0.59±0.02^{b}	0.81±0.01^{a}
Cu	<0.50*	<0.50*	<0.50*
Cd	<0.0040*	<0.0040*	<0.0040*
As	<0.030*	<0.030*	<0.030*
Hg	<0.002*	<0.002*	<0.002*
Ni	<0.10*	<0.10*	<0.10*
Pb	<0.10*	<0.10*	<0.10*

SD- standard deviation; ^{a,b,c} Means within a row with different superscripts differ (*P*<0.05); *Concentrations with this index are below the LOQ (limit quantification)

A benefit of presented study is correlation analysis of interaction between selected risk elements. Since the concentrations of toxic elements were found below the LOQ, correlations were performed only between the risk elements in sheep´s milk. In case of statistically significant correlations, there were mainly strong positive or negative correlations (Table 5). Strong positive correlation of relationships were found during the early lactation stage between Ca and Mg (r=0.920), Fe and Se (r=0.975). In the middle lactation stage, strong positive correlation was recorded between Ca and Mg (r=0.993), Ca and Se (r=0.998) and strong negative correlations between Ca and Fe (r= -0.962), Mg and Fe (r= -0.967), Se and Fe (r= -0.920). During the late lactation stage, strong negative correlations between Ca and Mg (r= -0.956), Mg and Zn (r= -0.995) and strong positive correlation in case of Ca and Zn (r= 0.966) were found. All mentioned correlations between the essential elements were statistically significant at the level P<0.01. As reported authors **Rodríguez Rodríguez *et al.* (1999)**, analyzes of correlation relationships between elements in milk are described very rarely in the literature. Interaction between liver, kidneys and muscle elements are more frequently described in the available literature compared to the milk (**Blanco-Penedo *et al.*, 2006; Issac *et al.*, 2012**). According to **Alonso *et al.* (2004)** it is caused by the fact, that these organs play the main role in trace element metabolism. The largest number of significant correlations between toxic and essential elements was found in the kidneys followed by liver (**Tomza-Marciniak *et al*., 2011**). Correlation analysis in present study showed similar strong positive correlation between Ca and Mg as stated by Rodríguez Rodríguez ***et al.* (1999)** (r=0.873) and **Pilarczyk *et al*. (2013)** in cow´s milk (r=0.89). Presented study also shows the higher value of correlation coefficient between Mg:Zn in early lactation stage and between Ca:Se and Mg:Se in the middle lactation stage compared to the sheep´s milk of Dubrovačka ruda breed in Croatia (**Antunović *et al*., 2016**).

Table 5 Significant Pearsons´s correlation analysis between elements in sheep´s milk during different lactation stages

Early lactation stage		Middle lactation stage		Late lactation stage	
Elements	**Correlation**	**Elements**	**Correlation**	**Elements**	**Correlation**
Ca:Mg	0.920**	Ca:Mg	0.933**	Ca:Mg	-0.956**
Ca:Zn	0.59	Ca:Zn	-0.67	Ca:Zn	0.966**
Ca:Se	0.005	Ca:Se	0.998**	Ca:Se	0.27
Ca:Fe	0.008	Ca:Fe	-0.962**	Ca:Fe	-0.464
Mg:Zn	0.622	Mg:Zn	-0.059	Mg:Zn	-0.995**
Mg:Se	0.006	Mg:Se	0.955**	Mg:Se	-0.265
Mg:Fe	0.006	Mg:Fe	-0.967**	Mg:Fe	0.626
Zn:Se	0.585	Zn:Se	-0.923	Zn:Se	0.659
Zn:Fe	0.588	Zn:Fe	0.690	Zn:Fe	-0.310
Fe:Se	0.975**	Se:Fe	-0.920**	Se:Fe	-0.119

**Correlation is significant at the P<0.01

CONCLUSION

The present study confirmed that the environment in the monitored area of Slovakia is in good quality and based on the actual environmental contamination can be this area therefore classified as an area with undisturbed environment. The study pointed out changes in the concentrations of risk and toxic elements in system soil-feed-milk and the detected concentrations of toxic elements in soil were not reflected in the samples of feed and milk of sheep. The influence of the lactation phase on changes in the concentrations of monitored elements in individual stages of lactation was confirmed. A comprehensive analysis of soil, feed and sheep milk in monitored area in relation to food control revealed that milk samples from monitored area did not contain concentrations of toxic elements and therefore it can be concluded that milk from selected area is in good hygienic quality and it is suitable for direct consumption or further processing.

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