

THE OCCURRENCE OF ELEVEN ELEMENTS IN DAIRY COW'S MILK, FEED, AND SOIL FROM THREE DIFFERENT REGIONS OF SLOVAKIA

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ABSTRACT

The objective of this study was to measure the concentrations of eleven essential, potentially toxic and toxic elements (arsenic – As, calcium – Ca, cadmium – Cd, copper – Cu, iron – Fe, mercury – Hg, magnesium – Mg, nickel – Ni, lead – Pb, selenium – Se, zinc – Zn) in raw cow's milk (spring, summer, and autumn season), feed (spring and autumn season) and soil (spring season) from three different environments by routine methods in the certified testing laboratory. The samples were collected in the undisturbed region around Novot', the moderately disturbed region around Tulčák, and the strongly disturbed region around Čečejevce. The concentrations of all toxic elements (As, Cd, Hg, Ni, Pb) and two essential elements (Cu, Se) in milk were under the limits of quantification (LOQ) from all investigated areas and during all seasons. Concentrations of other elements in milk from the undisturbed and disturbed areas were significantly different, generally with the highest levels in summer. In soil samples, the significantly highest concentrations of Ca, Cu, Ni were found in a strongly disturbed area, Mg and As in moderately disturbed area, and Fe, Se, Zn, Hg, and Pb in an undisturbed area. Cadmium was under the LOQ. In feed, the concentrations of essential elements, except of Se, were higher in the autumn. The significantly highest concentration of As, Ni were recorded in a moderately disturbed area and Pb in the undisturbed area in both seasons. Cadmium and Hg were under the LOQ. Despite the higher level of some elements in soil (Fe, Mg, Ca) from all regions, there were not elevated concentrations of any element in feed or milk. The concentrations of all toxic elements in milk were under the permitted limits. Thus, the milk from all investigated areas was not contaminated with the elements posing a health risk for consumers and it is considered safe for human consumption.

Keywords: essential element; toxic metal; cow milk; feed; soil

INTRODUCTION

Milk is a well-known source of many compounds with a beneficial role in the human organism. It contains essential elements, vitamins, proteins, and other compounds important mainly for the children's health. However, except for these important compounds, it may contain also toxic or potentially toxic elements. **Hermansen et al. (2005)** analysed 45 trace elements and 6 macro elements (Ca, potassium – K, Mg, sodium – Na, phosphorus – P, sulphur – S) in cow milk. Authors found the differences between the organically produced milk and conventionally produced milk with elevated concentration of molybdenum (Mo) in organic milk, whereas the concentrations of barium (Ba), europium (Eu), manganese (Mn), and zinc (Zn) were significantly reduced compared with conventional milk. Differences in element concentrations based on animal species and regions reported **Zhou et al. (2017)**. Authors also found that the concentrations of elements in water and feed might contribute to those in milk. Toxic and potentially

toxic elements in raw milk were associated with those in feed and drinking water. Results of meta-analysis made by **Zwierzchowski and Ametaj (2018)** show that concentrations of Pb were above the minimum-risk level (MRL) in the milk samples from Brazil, Croatia, Egypt, Mexico, Nigeria, Palestine, Romania, Serbia, and Turkey. Moreover, organic dairy farms are characterized by lower concentrations of toxic heavy metals in whole raw milk compared with those from the conventional production system.

A large proportion of the total amounts of the elements contains casein fraction in cow's milk but not in humans (**Fransson and Lönnnerdal, 1983**). Lead and As were found in the cow's milk from areas irrigated with wastewater in Mexico with Pb concentrations above the maximum limit as set by Codex Alimentarius and the European Commission standards (**Castro-González et al., 2018**). **Castro-González et al. (2019)** warn that chronic heavy metal consumption in contaminated cow's milk can pose a serious

health risk for girls and children. The heavy metals in the milk had the following order $Zn > As > Pb > Cr > Cu > Ni$. Lead exceeded the Codex limits. Many of the metals occurring in the milk can cause cancer in humans. As, Cd, chromium (Cr), Pb, and Hg are considered systemic toxicants and are also classified as human carcinogens (Tchounwou et al., 2012). Epidemiological studies have shown that As exposure is associated with a variety of human cancer of the skin, lungs, bladder, liver. Exposure occurs primarily via drinking water, but dietary exposure can also be substantial (Zhou and Xi, 2018). Environmental quality and human activities (soil, water, river, industry, mining, and smelting) play a key role in the distribution of toxic metals in raw milk and contribute to Pb, As, and Cd contamination in animals and transfer to milk (Kazi et al., 2009; Zhou et al., 2019a). In bovine milk, Hg is associated with two protein fractions, caseins, and beta-lactoglobulin (Mata, Sanchez and Calvo, 1997). Mercury in cow milk samples in concentration 3.1 ng.g^{-1} found Najarnejhad and Akbarabadi (2013). Nickel was also found in cow milk and high concentrations of Ni in traditional farms compared to industrial farms could be attributed to the location of this industry in a rural area (Arianejad et al., 2015).

Milk may be an important source of essential elements. The iron content of cow's milk is about 0.5 mg.L^{-1} and is thus comparable to that of human milk (Ziegler, 2011). However, cow's milk and Ca inhibit nonheme Fe absorption (Domellöf et al., 2014). Copper is found in lower concentrations in milk. However, the concentrations of Cu in the meat and cow milk samples were higher than the maximum allowable concentration (MAC) of Cu in foods in Bangladesh (Shaheen et al., 2016). Even higher Cu concentrations were found in Croatia (Bilandžić et al.,

2011). Milk can significantly contribute to the dietary Se intake in human. Higher milk Se concentrations have been measured in the Northern Ireland, but its content in milk is affected by geographical location (O'Kane et al., 2018). Effect of parity, stage of lactation and breed on mineral composition of cow milk reported Manuelian et al. (2018). Milk of primiparous cows had greater Ca, Mg, K, and P contents than milk of multiparous cows. Holstein-Friesian produced the lowest concentrations of Ca, Mg, and P content. Jersey yielded milk with the greatest Ca and Mg content.

Due to the importance of milk consumption and its content of essential and toxic elements mainly for children's health, the aim of this work was to analyse and compare the occurrence of 11 elements in raw cow's milk, feed and soil samples from different areas of Slovakia based on the environmental regional classification.

Scientific hypothesis

The concentration of toxic and essential elements in cow's milk is affected by the environmental quality.

MATERIAL AND METHODOLOGY

Sample collection

The cow's milk, feed and soil samples were collected in three different areas of Slovakia based on the environmental regional classification (MESR and SEA, 2018) (Figure 1). The location around Novot' is considered as a region with an undisturbed environment, the second analysed area of Tulčík is considered as a region with a moderately disturbed environment, and the third area considered as a region with a strongly disturbed environment is located around Čečejevce.

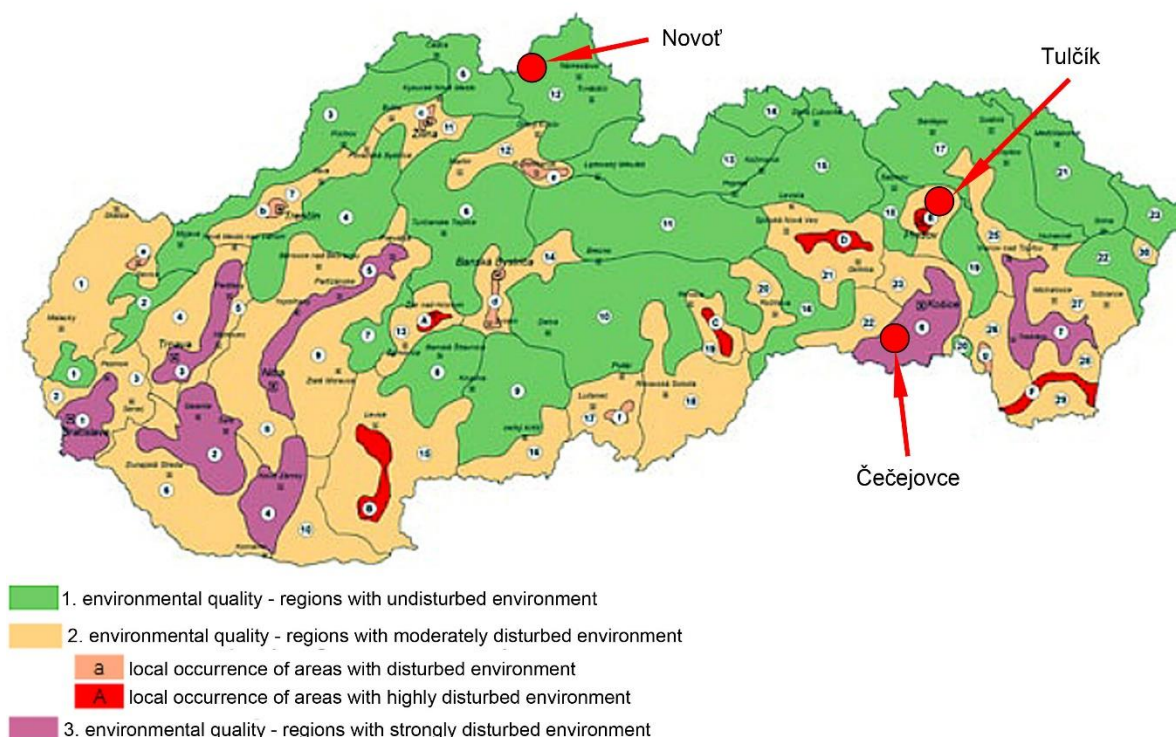


Figure 1 Environmental regional classification of Slovakia (MESR and SEA, 2018). Note: Three investigated regions (Novot', Tulčík, Čečejevce) are marked.

The tank milk samples were collected immediately after the cows were milked, in the morning and in the afternoon by an automatic milking system. Samples were collected three times a year; in spring (April), in summer (July), and in autumn (September). 500 mL of milk were collected directly from the milk tank during the two days (two times from morning milking and two times from afternoon milking). Five samples of milk were collected from each milking, in total 60 samples of pool milk (20 samples each season) from each location. The total number of the dairy cows were as follows: Novof' area (220 cows; crossbreeds: Slovak spotted breed × Red Holstein breed), Tulčik area (450 cows; Slovak spotted breed), and Čečejovce area (340 cows; Black Holstein breed). Average milk samples from these cows were obtained from milk tanks immediately after the end of milking. Samples were kept in PET bottles at -18 °C until analysis.

Ten feed samples (5 in April and 5 in September) of total mixed ration (TMR) were collected from each observed location. The feed was made at all farms from local components from these farms. Samples were stored in plastic bags at -18 °C until analysis.

Five soil samples were collected from different places at each farm during the spring season (April). Samples were stored in plastic bags at -18 °C until analysis.

Sample analysis

Milk samples for Ca, Fe, Mg, Cd, Ni, Pb, Cu, Zn analyses were mineralized by microwave decomposition with HNO₃ and H₂O₂ (microwave oven MARS 6 240/50). Milk samples for As analysis were prepared by dry mineralization with oxidation mixture (oxygen, oxides of nitrogen, ozone), heated at 300 – 400 °C. The ash was re-diluted in HCl solution. Milk samples for Se analysis were mineralized by microwave decomposition with HNO₃ and H₂O₂ (microwave oven MARS 6 240/50) and after removal of nitrous gases, cooling, and addition of HCl solution, Se⁶⁺ was reduced to Se⁴⁺ by heating at 90 °C.

Feed samples were prepared by dry mineralization in oven at 475 °C (Ca, Fe, Mg, Cu, Zn) or 580 °C (Cd, Ni, Pb) and the ash was diluted in HCl solution. Feed samples for As and Se analyses were prepared by magnesium nitrate and oxide suspension and mineralized with HNO₃ at 470 °C. The ash was diluted in the HCl solution. Se⁶⁺ was reduced to Se⁴⁺ by heating at 90 °C.

Soil samples for As, Se, Ca, Fe, Mg, Cd, Ni, Cu, Pb, Zn were extracted with aqua regia and cooled for 2 hours.

Arsenic and Se in milk, feed, and soil were analysed using the hydride generation atomic absorption spectroscopy (HG-AAS) method with SpectrAA-220 FS (The Netherlands). Calcium, Fe, and Mg in milk, feed, and soil samples were detected using the inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian 720-ES, USA). Cadmium, Pb, and Ni in milk and feed were analysed using the electrothermal atomization atomic absorption spectrometry (ETA-AAS, Agilent DUO AA 240Z/240FS, USA). Zinc and Cu in milk and feed and Cd, Ni, Cu, Pb, Zn in soil were analysed using the flame atomic absorption spectrometry (F-AAS, DUO AA 240Z/240FS, USA). Mercury in milk, feed, and soil samples was analysed using the Advanced Mercury Analyzer and atomic absorption spectrometry (AMA-AAS, AMA254, Altec, Czech Republic) without the need for chemical preparation of the sample. All analyses were conducted in certified testing laboratory Eurofins/Bel Novamann (Nové Zámky, Slovak Republic).

Quality assurance

For the validation of the analytical methods the limits of detection (LOD) and LOQ were evaluated (Table 1). LOD in digest was calculated as three times the standard deviation of the sample blank relative to the slope of the analytical curve. LOQ was calculated as 10 times the standard deviation of the sample blank relative to the slope of the analytical curve. LOD and LOQ were calculated separately for soil, feed, and for food in general. Based on the obtained LOD limits, LOQ limits according to the needs of **Commission Regulation (EC) no. 1881/2006** were obtained. LOQ limits were recalculated from mg.L⁻¹ (LOD) to mg.kg⁻¹ based on the sample weight and the final volume of the digest. The quality control (QC) during measurement was ensured by a parallel analysis of at least one sample and the method calibration was controlled before every measurement by the control sample from the calibration solution of certified reference material (CRM). The solution was prepared by diluting a standard solution (Ultra Scientific, USA) with a certified value of 1000 mg.L⁻¹ (multi-element solution).

Table 1 LOD and LOQ values for essential and toxic elements analysed.

Element	LOD (mg.kg ⁻¹)			LOQ (mg.kg ⁻¹)		
	milk	feed	soil	milk	feed	soil
Ca	2.0	2.0	2.3	6.0	6.0	7.0
Cu	0.017	0.17	0.3	0.05	0.50	1.0
Fe	0.17	0.17	3.0	0.50	0.50	10.0
Mg	0.33	0.33	0.3	1.0	1.0	1.0
Se	0.0067	0.017	0.06	0.03	0.050	0.20
Zn	0.17	0.17	2.0	0.50	0.50	6.0
As	0.01	0.017	0.06	0.03	0.050	0.20
Cd	0.0013	0.0067	0.13	0.0040	0.10	0.40
Hg	0.00067	0.0033	0.003	0.002	0.010	0.010
Ni	0.03	0.033	0.8	0.10	0.10	2.5
Pb	1.0	0.033	1.0	0.01	0.10	3.0

A standard from a different supplier was used to prepare the QC sample, and then the standard was used to prepare the calibration solutions.

The accuracy of the method, CRM, skimmed milk powder (ERM-BD151) was analysed for the determination of elements in milk, CRM, poultry feed-proximates and elements (LGC7173) and pig feed (BCR-709) for elements in feed and quality control material (CQM) river sediment (Metranal TM 1) for elements in soil samples.

Statistical analysis

Statistical analysis of the data was performed using SAS 9.2 (SAS Institute Inc., USA). Differences in concentrations of the analyzed elements in feed, soil, and cow's milk between seasons and three investigated areas were compared by the ANOVA and Student's *t*-test. All data were expressed as mean, standard deviation, and coefficient of variation. A probability level of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Except of Cu and Se, all analysed essential elements were present in all milk samples (Table 2). The significantly highest Ca concentration was found in the undisturbed area of Novot' in spring and summer in comparison to the other two areas. Significantly lowest Ca concentration in spring was found in milk from the Tulčik area ($p < 0.01$), in summer and autumn in the Čečejevce area ($p < 0.001$ and $p < 0.05$, respectively). The effect of season on Ca concentration in cow milk is contradictory in the literature. **Lin et al. (2017)** reported that season had no significant effect on total Ca in milk. Also, **Chassaing et al. (2016)** did not confirm the seasonal changes of mineral contents in cow milk. The concentrations of Ca in cow milk vary and ranged from 1203.5 to 1316.1 mg.kg⁻¹ (**Toffanin et al., 2015**), 1043 – 1283 mg.kg⁻¹ (**Gaucheron, 2005**), 1409.86 – 2156.45 mg.kg⁻¹ (**Pilarczyk et al., 2013**) up to 3789.7 mg.L⁻¹ (**Capcarova et al., 2019**), and in our analyses, they ranged from 981 mg.kg⁻¹ (Tulčik in spring) to 1930 mg.kg⁻¹ (Novot' in summer). Moreover, **Toffanin et al. (2015)** found that Ca milk content decreased between March and May. Similar trends in Ca milk concentration were found in our analyses and are supported by findings of **Hurtaud et al. (2014)** and **Poulsen et al. (2015)**. **Boudon et al. (2016)** suggest that long and sunny days could explain part of the seasonal decrease in milk Ca content in summer.

Significantly lowest Fe concentration in milk was found in the Čečejevce area in spring ($p < 0.01$) and in autumn ($p < 0.05$). Significantly lowest Mg concentration was found in Tulčik in spring and summer ($p < 0.001$) and in Čečejevce in autumn ($p < 0.01$). The highest Mg concentration was found in Tulčik during the autumn season ($p < 0.001$) in comparison to the undisturbed area of Novot', where the Mg concentration was lowest in autumn. The same and lowest Zn concentrations in milk were recorded around Tulčik and Čečejevce with a significant decrease in spring ($p < 0.001$). In summer, the lowest Zn concentration was found in the Tulčik area ($p < 0.01$) and in autumn in the Čečejevce area ($p < 0.01$). In the previous study, **Pšenková et al. (2020)** detected in the strongly disturbed environment of the Čečejevce area in 2016 only Ca, Mg, and Zn in the cow

milk. In our study, the Ca, Mg, and Zn concentrations were about 2 – 3 times higher. High level of variability in the Ca, Cu, Fe, Na, Ni, and Zn contents in cow milk found **Capcarova et al. (2019)** in Slovakia (SK) and Czech Republic (CZ). The concentrations of observed elements in SK and CZ milk built an order of increasing concentrations: Ni < Cu < Fe < Zn < Mg < Na < K < Ca. The same trend was observed in our experiments, no matter the season and area. A similar concentration of Zn in cow milk was found by **Wiking, Larsen and Sehested (2008)**, **van Hulzen et al. (2009)**, **Stocco et al. (2019)**. The Mg concentrations in milk were in the normal range 97 – 146 mg.kg⁻¹ as described **Gaucheron (2005)** with slightly higher levels in summer (154 – 164.2 mg.kg⁻¹) in all areas. Similar results recorded **Pilarczyk et al. (2013)** at an organic farm in Poland. The concentrations of essential elements in milk from all areas were in the normal range meaning there was no obvious contamination. A different situation was recorded in Egypt. The analyses of Fe (16.38 mg.kg⁻¹), Zn (10.75 mg.kg⁻¹), and Cu (2.83 mg.kg⁻¹) showed that most of the cow milk samples from the different sites contained all the studied metals with a concentration higher than those recommended for milk by the international dairy federation standard and Codex (**Malhat et al., 2012**). The element with the highest frequency of occurrence in cow milk in Turkey was Zn, followed by Cr = As > Al > Se > Fe > Ni > Cu > Pb = Cd, in decreasing order. The lowest concentration among the essential elements was seen in Cu. Al and As were very often found but Pb and Cd were not found in the milk samples (**Totan and Filazi, 2020**). In our samples, the lowest concentration of the essential elements was found in Fe in the range of 0.404 – 0.626 mg.kg⁻¹ aside from the season and area. On the contrary, average concentrations of Fe in all samples of milk analyzed in Palestine were the highest (2.01 – 3.86 mg.kg⁻¹) (**Abdulkhalik et al., 2012**). In our study, the concentrations of toxic and potentially toxic elements (As, Cd, Ni, Hg, Pb) in cow milk from all analyzed areas and in any season were under the LOQ (Table 1). It is important information due to the toxic nature of these elements. The milk from the investigated areas was not contaminated with the elements posing a health risk for consumers and it is considered safe for human consumption. In contrast, **Pilarczyk et al. (2013)** found Pb content in the milk of cows of two breeds two times higher than the permissible concentration of 0.02 mg.kg⁻¹ in the raw milk given by the standards of the **Commission Regulation (EC) no. 1881/2006**. **González-Montaña et al. (2019)** also found some of the metals in milk (aluminium – Al, As, Mo) in the area with various anthropogenic activities (industrial, mining, traffic density). **Datta et al. (2012)** warned that consumption of milk from the contaminated areas might have produced arsenicosis and may be considered as an alternative source of arsenic contamination. Milk samples collected from the nonindustrial region of Turkey in the summer had higher Cr, Mn, and Zn concentrations than the polluted region. However, industrial activities and seasonal changes had no significant effect on selected element concentrations on cow milk (**Erdogan, Celik and Erdogan, 2004**). **Qu et al. (2018)** found relative high toxic metal levels from provinces with heavy industry. The average of milk exposure concentration for As, Pb, Cr, Hg, Al, and Ni was 1.35, 8.50, 34.58, 2.31, 284.16, and 10.78 µg.L⁻¹, respectively. Ni in milk is accumulated in fat.

This probably essential element was found in cow milk from Turkey in a concentration of 8.71 $\mu\text{g}\cdot\text{kg}^{-1}$ (Totan and Filazi, 2020) and from Spain 4 – 25 $\mu\text{g}\cdot\text{kg}^{-1}$ (Llorent-Martínez et al., 2012).

The occurrence of contaminants in animal milk is connected with environmental quality. Metals are transferred from soil to water and/or feed to milk. Zhou et al. (2019b) note that different kinds of heavy metal contamination in raw milk may travel through complex pathways from the environment, directly or indirectly, via drinking water and soil. Heavy metals in silage may be the main contributor to milk contamination, as Pb, As, Cr, and

Cd in silage all showed positive correlations with those in milk. The authors found that water may be the source of Pb and As in the milk, while Cr and Cd are transferred from the soil. The levels of elements in soil samples are shown in Table 3. The highest level of Ca, Cu, and Ni in soil was recorded in the strongly disturbed environment of the Čečejevce area. The source of metals may be an industrial activity (steel production, mining, waste combustion). The concentrations of Pb, Hg, Cd, As, Cu in the soil in this area exceeded the maximum limits in 2016 (Juhosová et al., 2017). However, the concentrations of those metals

Table 2 Concentrations of essential and toxic elements in cow's milk in spring, summer and autumn in different environment.

Element	Undisturbed area ($\text{mg}\cdot\text{kg}^{-1} \pm \text{SD}$)	Moderately disturbed area ($\text{mg}\cdot\text{kg}^{-1} \pm \text{SD}$)	Strongly disturbed area ($\text{mg}\cdot\text{kg}^{-1} \pm \text{SD}$)
Spring season (April)			
Ca	1294 \pm 11.402	981 \pm 92.898 ^{a**}	1098 \pm 98.590 ^{b*}
Cu	<0.05 ¹	<0.05 ¹	<0.05 ¹
Fe	0.482 \pm 0.013	0.468 \pm 0.019	0.404 \pm 0.034 ^{b** c**}
Mg	114 \pm 2.646	85.4 \pm 5.550 ^{a***}	95.6 \pm 3.578 ^{b*** c**}
Se	<0.03 ¹	<0.03 ¹	<0.03 ¹
Zn	4.24 \pm 0.195	3.44 \pm 0.182 ^{a***}	3.44 \pm 0.270 ^{b***}
As	<0.03 ¹	<0.03 ¹	<0.03 ¹
Cd	<0.004 ¹	<0.004 ¹	<0.004 ¹
Hg	<0.002 ¹	<0.002 ¹	<0.002 ¹
Ni	<0.1 ¹	<0.1 ¹	<0.1 ¹
Pb	<0.01 ¹	<0.01 ¹	<0.01 ¹
Summer season (July)			
Ca	1930 \pm 35.355	1426 \pm 160.873 ^{a**}	1570 \pm 93.005 ^{b***}
Cu	<0.05 ¹	<0.05 ¹	<0.05 ¹
Fe	0.626 \pm 0.018	0.66 \pm 0.045	0.606 \pm 0.030
Mg	164.6 \pm 2.302	154 \pm 1.225 ^{a***}	160.2 \pm 7.014
Se	<0.03 ¹	<0.03 ¹	<0.03 ¹
Zn	5.86 \pm 0.114	4.8 \pm 0.245 ^{a***}	5.48 \pm 0.460 ^{c*}
As	<0.03 ¹	<0.03 ¹	<0.03 ¹
Cd	<0.004 ¹	<0.004 ¹	<0.004 ¹
Hg	<0.002 ¹	<0.002 ¹	<0.002 ¹
Ni	<0.1 ¹	<0.1 ¹	<0.1 ¹
Pb	<0.01 ¹	<0.01 ¹	<0.01 ¹
Autumn season (September)			
Ca	1178 \pm 42.071	1214 \pm 25.010	1130 \pm 50.990 ^{c*}
Cu	<0.05 ¹	<0.05 ¹	<0.05 ¹
Fe	0.482 \pm 0.019	0.482 \pm 0.066	0.428 \pm 0.041 ^{b*}
Mg	92.8 \pm 3.114	103.8 \pm 2.950 ^{a***}	97 \pm 3.162 ^{c**}
Se	<0.03 ¹	<0.03 ¹	<0.03 ¹
Zn	3.36 \pm 0.089	4.42 \pm 0.383 ^{a**}	3.64 \pm 0.195 ^{b* c**}
As	<0.03 ¹	<0.03 ¹	<0.03 ¹
Cd	<0.004 ¹	<0.004 ¹	<0.004 ¹
Hg	<0.002 ¹	<0.002 ¹	<0.002 ¹
Ni	<0.1 ¹	<0.1 ¹	<0.1 ¹
Pb	<0.01 ¹	<0.01 ¹	<0.01 ¹

Note: SD – standard deviation; ^a differences between undisturbed area and moderately disturbed area; ^b differences between undisturbed area and strongly disturbed area; ^c differences between moderately disturbed area and strongly disturbed area; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ¹ Values below LOQ (limit of quantification).

recorded in our analyses were much lower or not detected (Cd).

Cd was also not detected in any of the milk samples collected dairies in the proximity of mines in Gauteng and North West Provinces of South Africa. This indicates the absence of Cd related toxicological risks in studied dairy farms (Ataro et al., 2008). Arsenic concentration in soil samples was significantly highest ($p < 0.001$) in the Tulčik area, Hg and Pb were found highest ($p < 0.001$) in the Novot' area, Ni was significantly highest ($p < 0.001$) in the Čečejevce area. The detailed biological cycle which causes the uptake of trace elements from the feed into milk or other dairy products is not well understood (Herwig et al., 2011). Rey-Crespo et al. (2013) assume that the significantly higher As (65%) and Fe (13%) concentrations in cow milk found in the winter is probably related to higher consumption of concentration feed and soil ingestion when grazing. Zhou et al. (2017) also note that toxic and potentially toxic elements in raw milk were associated with those in feed and drinking water. Trace elements Mn, Fe, Ni, Ga, Se, Sr, Cs, U in water and Co, Ni, Cu, Se, U in feed were significantly correlated with those in milk. Moreover, the toxic and potentially toxic elements Cr, As, Cd, Tl, Pb in water and Al, Cr, As, Hg, Tl in feed were significantly correlated with those in milk. An analysis of cow's feed and milk at wastewater-irrigated agricultural farms in Pakistan revealed that contaminated fodder like maize and Brassica plants grown with wastewater and contaminated soil are common sources contributing the heavy metal contamination in raw milk (Iqbal et al., 2020). Pb, Fe, Cu, and Zn were higher in the milk samples collected from the industrial region around Bursa, a province of Turkey, but no Hg was detected (Simsek et al., 2000). Surprisingly, the TMR feed from strongly disturbed area contained significantly lowest concentrations ($p < 0.001$) of all detected toxic and potentially toxic elements (As, Ni, Pb) in our analyses in spring. In autumn, the concentrations of these metals in feed were slightly higher in the Čečejevce area than in spring, but not the highest among the investigated areas. On the contrary, the „cleanest“ area, officially classified as an area with an undisturbed environment, contained significantly highest ($p < 0.001$)

levels of As in spring and Pb ($p < 0.001$) in spring and autumn in the feed. The occurrence of toxic metals in soil and feed did not affect their levels in cow milk as their concentrations in milk from all observed areas were under the LOQ. The concentrations of As in soil decreased in the order: moderately disturbed > undisturbed > strongly disturbed area. The same order was recorded in the feed in spring. The concentrations of Pb in soil and feed decreased in the same order: undisturbed > moderately disturbed > strongly disturbed area. The Ni concentration in soil in spring decreased in the order: strongly disturbed > undisturbed > moderately disturbed area, but in the feed, it decreased as follows: undisturbed = moderately disturbed > strongly disturbed area. The Hg concentration in soil in spring decreased in the order: undisturbed > strongly disturbed > moderately disturbed area but Hg in feed was under LOQ both in spring and autumn. These results confirm the variability of feed and soil contamination in different areas. Variable concentrations of Al, As, Ni, Hg, Pb, and Cd among most of the investigated regions of Egypt reported Diab et al. (2020). The reason for these differences is probably different soil accumulation characteristics for different metals. Different soil accumulation characteristics for Cd, Cu, Zn, Pb, Ni, and Cr found Zhang et al. (2018). Anyway, Vidovic et al. (2005) found a direct influence of atmospheric deposits on Cd and Zn distribution in the chain soil-cattle feed-milk. A significant decrease of the Cd and Zn concentrations in atmospheric deposits, originated mostly from vehicle traffic, resulting in a decrease of these metals in cow's feeds, milk, and soil in the Kikinda region, Serbia.

The concentrations of elements in feed analysed in three different regions are summarized in Table 4. Significant increases in milk Se concentration were observed with an increasing level of Se in the diet of cows and thus the concentration of Se in bovine milk is related to Se concentration in the feed (Givens et al., 2004; Haug, Høstmark and Harstad, 2007). The Se content in the soil in Slovakia and Central Europe is generally low (Ducsay, Ložek and Varga, 2009; Sager, 2006) but there is a difference between the regions and soil types.

Table 3 Concentrations of essential and toxic elements in soil in spring in different environment.

Element	Undisturbed area (mg.kg ⁻¹ ±SD)	Moderately disturbed area (mg.kg ⁻¹ ±SD)	Strongly disturbed area (mg.kg ⁻¹ ±SD)
Spring season (April)			
Ca	3930 ±45.826	3688 ±101.833 ^{a**}	10652 ±1015.071 ^{b*** c***}
Cu	25.92 ±0.853	16.54 ±1.029 ^{a***}	30.02 ±1.619 ^{b** c***}
Fe	26010 ±441.814	22908 ±203.887 ^{a***}	17840 ±392.747 ^{b*** c***}
Mg	4692 ±81.670	5102 ±68.337 ^{a***}	4490 ±70.0 ^{b** c**}
Se	0.308 ±0.008	0.242 ±0.037 ^{a*}	0.18 ±0.012 ^{b*** c*}
Zn	106.4 ±4.278	63.82 ±5.265 ^{a***}	59.9 ±1.061 ^{b***}
As	5.54 ±0.251	8.84 ±0.477 ^{a***}	3.32 ±0.148 ^{b*** c***}
Cd	<0.4 ¹	<0.4 ¹	<0.4 ¹
Hg	0.069 ±0.002	0.049 ±0.002 ^{a***}	0.065 ±0.003 ^{b* c***}
Ni	21.54 ±0.329	20.88 ±0.335 ^{a*}	34.96 ±0.902 ^{b*** c***}
Pb	27.64 ±0.503	17.58 ±0.526 ^{a***}	15.7 ±0.406 ^{b*** c***}

Note: SD – standard deviation; ^a differences between undisturbed area and moderately disturbed area; ^b differences between undisturbed area and strongly disturbed area; ^c differences between moderately disturbed area and strongly disturbed area; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ¹ Values below LOQ (limit of quantification).

In Slovakia, the Se content in soil ranges from 0.04 to 0.80 mg.kg⁻¹ but in the Nitra region, Se soil concentration exceeded the limit value 1.25-times (Hegedusova et al., 2016). Our results (0.18 – 0.308 mg.kg⁻¹) show that Se in soil from all observed regions was in the range of average levels of Se in Slovakia. Very low (under the LOQ) concentrations of Se in milk is also caused by the low transfer of Se from soil to feed. In TMR feed, the Se concentrations were about 10-times lower than that in the soil in spring and slightly higher (up to 0.336 mg.kg⁻¹) in autumn. A similar trend was recorded in Cu concentration. Despite the fact, that Cu was under the LOQ in milk, we have found Cu in soil and feed samples from all investigated areas. The significantly highest Cu levels in soil were found in the strongly disturbed area in comparison to moderately disturbed ($p < 0.001$) and undisturbed area ($p < 0.01$). In feed, the significantly highest Cu concentration ($p < 0.001$) was found in a moderately disturbed environment in spring and autumn in comparison to the undisturbed area. The limit value for soil varies by the soil type from 30 to 70 mg.kg⁻¹ (Regulation no. 508/2004). The concentration of Cu in the soil in Slovakia is 17 mg.kg⁻¹ and at the cinnabar mine site, it ranges from 20.7 to 24.9 mg.kg⁻¹ (Kulikova et al., 2019). Our results are comparable to these values found in the country.

Soil Fe levels are not limited in Slovakia. Relatively high Fe concentrations were found in soil samples from all observed areas with significantly highest ($p < 0.001$)

concentration in the undisturbed area. Khan et al. (2011) reported a non-significant effect of sampling periods on soil Fe content, however, a higher transfer of Fe to pastures was found during October. Iron feed levels were higher in autumn in comparison to spring samples. The significantly highest ($p < 0.001$) concentrations were found in the undisturbed area in spring and a moderately disturbed environment in autumn. Soil-derived elements like Fe may be ingested during grazing (Orjales et al., 2018) but we did not find an increase in Fe milk concentration above the normal levels.

Concentrations of Mg in soil did not differ between the spring and fall and ranged depending on the depth from 390 to 426 mg.kg⁻¹ (Hristov, Hazen and Ellsworth, 2007). About 10-times higher concentrations of Mg in soil samples were found in our study. The significantly highest ($p < 0.001$) Mg level in soil was recorded in the moderately disturbed area in comparison to the undisturbed area of Novot'. Much higher concentrations of Mg (33.8 – 38.4 g.kg⁻¹) were found in contaminated soil at a magnesite mining region in China (Wang et al., 2015). Concentrations of Mg in the soil are not limited in Slovakia. The feed contained generally higher Mg levels in autumn with the significantly highest levels in the moderately disturbed area which is in accordance with Mg levels in the soil. Plant Mg content in plant feeds varies between 0.7 and 3 g.kg⁻¹ (Haaranen, 2003). Our analyses show that the cow's feed contained around 3 g.kg⁻¹.

Table 4 Concentrations of essential and toxic elements in feed in spring and autumn in different environment.

Element	Undisturbed area (mg.kg ⁻¹ ±SD)	Moderately disturbed area (mg.kg ⁻¹ ±SD)	Strongly disturbed area (mg.kg ⁻¹ ±SD)
Spring season (April)			
Ca	2574 ±23.022	8412 ±397.706 ^{a***}	4844 ±367.872 ^{b*** c***}
Cu	4.36 ±0.207	12.3 ±0.667 ^{a***}	11.08 ±0.763 ^{b*** c*}
Fe	263 ±3.464	215 ±10.977 ^{a***}	223.8 ±18.226 ^{b**}
Mg	1558 ±14.832	3692 ±97.570 ^{a***}	1232 ±92.033 ^{b** c***}
Se	0.062 ±0.009	0.086 ±0.005 ^{a***}	0.021 ±0.003 ^{b*** c***}
Zn	30.82 ±1.711	83.26 ±1.358 ^{a***}	51.46 ±1.539 ^{b*** c***}
As	0.088 ±0.001	0.13 ±0.024 ^{a*}	0.025 ±0.003 ^{b*** c***}
Cd	<0.1 ¹	<0.1 ¹	<0.1 ¹
Hg	<0.01 ¹	<0.01 ¹	<0.01 ¹
Ni	1.48 ±0.084	1.48 ±0.130	0.192 ±0.042 ^{b*** c***}
Pb	0.56 ±0.012	0.506 ±0.042 ^{a*}	0.236 ±0.048 ^{b*** c***}
Autumn season (September)			
Ca	9938 ±470.394	7964 ±198.570 ^{a***}	10354 ±116.103 ^{c***}
Cu	12.82 ±1.132	25.98 ±1.043 ^{a***}	20.78 ±0.512 ^{b*** c***}
Fe	537.2 ±14.653	832 ±44.385 ^{a***}	441.8 ±31.444 ^{b*** c***}
Mg	3082 ±75.961	3308 ±21.679 ^{a**}	2952 ±101.341 ^{c**}
Se	0.053 ±0.003	0.25 ±0.029 ^{a***}	0.336 ±0.019 ^{b*** c***}
Zn	57.04 ±1.278	128 ±1.871 ^{a***}	127.8 ±3.114 ^{b***}
As	0.153 ±0.040	0.184 ±0.023	0.166 ±0.015
Cd	<0.1 ¹	<0.1 ¹	<0.1 ¹
Hg	<0.01 ¹	<0.01 ¹	<0.01 ¹
Ni	1.42 ±0.084	3.14 ±0.321 ^{a***}	1.82 ±0.192 ^{b** c***}
Pb	0.48 ±0.012	0.308 ±0.019 ^{a***}	0.39 ±0.016 ^{b*** c***}

Note: SD – standard deviation; ^a differences between undisturbed area and moderately disturbed area; ^b differences between undisturbed area and strongly disturbed area; ^c differences between moderately disturbed area and strongly disturbed area; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ¹Values below LOQ (limit of quantification).

These concentrations agree with the soil Mg levels and did not cause the elevation of Mg in raw cow milk.

Zn concentration in bovine milk is significantly affected by the dietary intake of fat and the transfer of fat from diet to milk might facilitate the transfer of Zn from diet to milk. (Wiking, Larsen and Sehested, 2008). The authors also found a similar trend in the variability of Zn levels in the feed as we have found in our analyses. The significantly highest ($p < 0.001$) Zn concentration in feed was found in spring and autumn in a moderately disturbed area and in a strongly disturbed area. These findings may correspond to the mining and industrial (metallurgy) activities in these areas and using of pesticides containing metals like Zn. Mean Zn concentrations in soil in the cinnabar mining site in Slovakia found by Kulikova et al. (2019) ranged from 58.1 to 61.6 mg.kg⁻¹, which are comparable to levels in disturbed areas in our study. However, the limit value for soil Zn by the soil type ranges from 100 to 150 mg.kg⁻¹ (Regulation no. 508/2004) and our results show Zn levels in the soil below the lowest permitted limit in disturbed areas. On the contrary, the significantly highest ($p < 0.001$) Zn concentration in soil was found in the undisturbed area. The concentrations of Zn in soil and feed do not correspond. Lower levels of Zn in soil recorded Khan et al. (2006) in Pakistan, but Baranowska, Barchańska and Pyrsz (2005) found Zn content in the soil in the higher range of 9.15 – 424.5 µg.g⁻¹ in Poland.

CONCLUSION

The positive findings of this study are, that the concentrations of all toxic elements in milk were under the limits of quantification from all investigated areas regardless of the environmental contamination level. We found seasonal variations in occurrence of essential elements with the highest levels in summer. The significantly highest levels of some elements in soil were recorded in the undisturbed environment. In feed, the higher concentrations almost all elements were found in the autumn. Higher levels of some elements in soil did not cause their elevation in the feed and milk. The milk from the investigated areas was not contaminated with the elements posing a health risk for consumers and it is considered safe for human consumption. Despite the fact, that all concentrations of analyzed elements were under the permissible limits, there is a constant need to monitor an environmental burden of metals in the different, even undisturbed regions of Slovakia with animal production to recognize another, hidden sources of metal contamination that may impact the food chain and human health.

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