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Author: Martina Pšenková, Róbert Toman, Vladimír Tančín,

Simona Almášiová, Ivona Jančo

Slovak University of Agriculture in Nitra, Slovakia

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CONCENTRATIONS OF SELECTED ESSENTIAL AND TOXIC ELEMENTS IN COW'S MILK AS A RAW MATERIAL WITH SIGNIFICANT PROCESSING POTENTIAL FROM AREAS WITH DIFFERENT ENVIRONMENTAL BURDEN

¹Martina Pšenková, ²Róbert Toman, ³Vladimír Tančín, ⁴Simona Almášiová, ⁵Ivona Jančo
^{1,2,3,4}*Slovak University of Agriculture in Nitra, Faculty of Agrobiological Sciences, Institute of Animal Husbandry, 5*⁵*Research Centre ABT CPU in Nitra, Slovakia*

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. Nutritional value, accessibility and variability of usage make milk a significant part of the human diet and a very good raw material for further food and industrial processing. Regular consumption of milk brings a lot of positive health effects. However, due to environmental pollution, milk may contain also toxic elements with adverse effects. Therefore, the aim of this study was to analyze of 10 essential and toxic elements (Ca, Na, K, Mg, Zn, Fe, Al, Li, Ni, Sr) in raw cow milk from 3 areas of Slovakia with different environmental burden: Zamagurie – undisturbed environment, Poiplie (Novohrad) – slightly disturbed environment, Podunajsko – strongly disturbed environment. From all monitored elements, concentrations of Ni were below the LOD limits. Mean concentrations of Al, Li and Sr was very low and differences in concentrations between areas was not statistically significant. From essential elements, the highest concentrations were found in case of Ca and Na and statistically significant differences ($P < 0.05$) was detected between undisturbed and slightly disturbed area of Slovakia in case of Ca, Na, K and Mg. Due

to very low concentrations of toxic elements or below the LOD limit, cow's milk from monitored areas is considered safe for human consumption and for further food or industrial processing.

Keywords: milk, food processing, elements, environmental pollution, quality

INTRODUCTION

Milk and dairy products are an important source of nutrients in human nutrition and is a source of energy, high quality proteins and essential vitamins and minerals. Milk is obtained from cows, sheep, goats, buffaloes, mares, camels, lamas, and reindeers Milk is composed of more than 100 components and the basic components of milk are fat, proteins, lactose, minerals, vitamins, non- protein nitrogenous substances and water. Milk is fluid consumed worldwide that is essential in the nutrition of millions of people around the world because it provides important macro- and micronutrients. Milk is considered an important part of the diet during childhood and adolescence due to its composition, but its relatively high content of saturated fats increases the risk of potential adverse effects, such as the risk to the cardiovascular system (Visioli and Strata, 2014). Since then, the consumption of milk and its use as a raw material for the preparation of food,

especially cow's milk, has emerged and spread to every inhabited continent and plays a key role in the diet of all people of all ages. Today's nutritional role of milk and milk-based products is defined by the FAO and other renowned governmental and academic groups in countries around the world. The nutritional recommendations are the result of a well-understood definition of milk and the standard composition of nutrients and their components. Nevertheless, the definition of milk has been examined several times in recent years, mainly due to the presence on the market of various dairy beverages of plant origin, where producers have chosen to use the name "milk" to explain a product made as an imitation and substitute for cow's milk (Moatsou and Sakkas, 2019).

Milk and dairy products are a complex system consisting of the coexistence of different nutrients, structural elements, and phases. Micro- and macrostructures and common processing processes may alter the interactions between nutrients in milk and dairy products because of a different metabolic response after consumption. Thus, the nutritional properties of dairy products should not be considered as the same as their nutrient equivalents in relation to different body functions and disease risks (Moatsou and Sakkas, 2019). According to information gathered in 2009, cow's milk provides almost 8% of energy, 12% of fat and almost 16% of protein in the diet of people in North America, with even higher percentages of the individual ingredients in Europe. In addition, dairy products provide 52-67% of the recommended nutritional intake of calcium in the diet of Americans. The carbohydrate contribution to the diet is less pronounced, although carbohydrates are usually the richest macronutrient. Lactose in cow's milk helps absorb and store many micronutrients in the intestine, such as calcium, magnesium, phosphorus, and

vitamin D (Chalupa-Krebdzdzak et al., 2018).

Many studies prove that regular consumption of milk and milk products has a beneficial effect, for example, in the prevention of metabolic syndrome (Lee et al., 2018), diabetes, bone health (Fardelone, 2019), supports sleep quality (Komada et al., 2020), reduces the risk of cancer (Aune et al., 2012; Zang et al., 2015), and helps preserve muscle mass even in old age (Hanach et al., 2019). The presence of elements in milk can have a physiological cause (elements are the building blocks of vitamins or enzymes), technological (transition of elements into milk as part of the production process - copper or iron) or toxicological (transfer of heavy metals from the environment) (Górska and Oprzadek, 2011). Mineral elements are essential for the animal organism and play an important role in metabolic processes such as maintaining pH, osmotic pressure, nerve conduction and muscle contraction (Bakircioglu et al., 2018). Inadequate intake of elements such as Ca, Mg, K, Na, whether insufficient or excessive, is associated with serious health problems such as hypertension, osteoporosis, cardiovascular diseases and others (Bilandžić, 2015). The trace elements found in milk have versatile uses in the human body, from the development of immunity to the provision of antioxidant capacity (Wang et al., 2014). Most trace metals, i.e., Cu, Zn, Mn, Co, and Fe are essential cofactors of many enzymes and basic physiological functions (Akele et al., 2017; Bakircioglu et al., 2018). The content of micro and macro elements in milk can vary significantly due to various factors such as the age of the dairy cow, the health of the animal, the lactation period, breeding, the season, the quality of the feed, the method of processing, fermentation, or possible fortification (Bakircioglu et al., 2018). However, the mentioned essential elements, which are necessary in small

quantities, in large quantities can also have toxic effects on human health, and their higher occurrence is mostly associated with environmental contamination (Kodrik et al., 2011; Kaptan et al., 2016).

In addition to essential elements, milk may also contain toxic elements such as aluminum, arsenic, cadmium, lead, strontium, some of which may also have mutagenic potential (Toman et al., 2021). Heavy metals pose a risk especially to children because, unlike adults, they are exposed to these substances through breast milk, the placenta and receive them accidentally by putting objects in the mouth, they have a higher rate of basal metabolism and a lower ability to metabolize toxic substances. In addition, their organs are more sensitive to foreign substances (Zeng et al., 2016). The combined effect of metals found in cow's milk and subsequently in dairy products poses a serious risk to children in contaminated areas (Castro Gonzales et al., 2017). Considering the importance of milk and mineral substances in human nutrition and the toxicity and impact of toxic elements on human and animal health, the aim of this study was to compare the levels of selected chemical elements in cow's milk from farms originating from areas with different environmental loads and to assess whether it has a higher environmental load, influence on the concentrations of monitored elements.

METHODOLOGY

In 2020 and 2021, during the following 12 months, cow's milk was collected from milk tanks from selected farms from various environmentally burden areas of Slovakia. A total of 36 milk samples were taken, that is, 12 milk samples from each selected farm. According to the Slovak Environmental Agency, which is part of the Ministry of the Environment of the Slovak Republic (2021), the selected areas

of this study are characterized by the following levels of environmental status: the first area – Zamagurie – undisturbed environment, the second area – Poiplie (Novohrad) – slightly disturbed environment, the third area – Podunajsko – strongly disturbed environment (Figure 1).

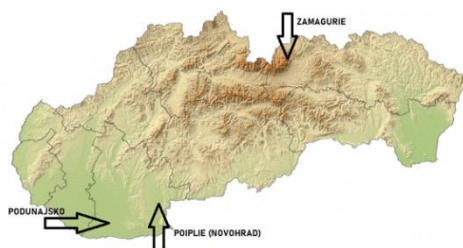


Figure 1 - Map of Slovakia and location of monitored areas

All the selected farms are based on a conventional farming method and the farms use cattle for milk production – Slovak spotted cattle (Slovak national breed).

A total of 10 chemical elements were monitored in the milk samples (essential elements – Ca, Na, K, Mg, Zn, Fe; toxic elements – Al, Li, Ni, Sr).

The content of selected elements was determined in samples of milk by inductively coupled plasma optical emission spectrometry (ICP-OES). As a first step, the pre-analytical procedure was made. All the chemicals used during the sample preparation were highly pure. Until the sample processing, they were stored in freezer at -20 °C. Weight of the experimental samples ranged from 0.5 to 2.0 g and was reflected in measurement. The samples were mineralized in the high-performance microwave digestion system Ethos UP (Milestone Srl, Sorisole, BG, Italy) in a solution of 5 mL HNO₃ ≥69.0% (TraceSELECT®, Honeywell Fluka, Morris Plains, USA), 1mL H₂O₂ ≥30%, for trace analysis (Sigma-Aldrich, Saint – Louis, Missouri, USA) and 2mL of

ultrapure water (18.2 MΩ cm-1; 25 °C, Synergy UV, Merck Millipore, France).

Samples, including the blank sample, were digested according to method for animal tissue developed and recommended by manufacturer to achieve the most reliable results. The method consists of heating and cooling phases. During the heating stage the samples were warmed to 200 °C for 15 min and this temperature was maintained for another 15 min. Afterwards, during the cooling phase, the samples underwent the active cooling for 15 min to reach the temperature of 50 °C. The digests were filtered through the VWR Quantitative filter paper 454 (particle retention 12 – 15 µm) (VWR

International, Leuven, France) into the volumetric flasks and filled up with ultrapure water to a volume of 50 mL. Analysis of the elements (Ag, Al, Ba, Ca, K, Li, Mg, Mo, Na, Sb, Sr, Zn) was carried out using inductively coupled plasma - optical emission spectrometer (ICP OES 720, Agilent Technologies Australia (M) Pty Ltd.) with axial plasma configuration and with auto-sampler SPS-3 (Agilent Technologies, Switzerland). The limits of detection (LOD) and limits of quantification (LOQ) were evaluated for the validation of the analytical method (Table 1).

Table 1 - LOD and LOQ values for analyzed elements in milk and dairy products (µg/kg)

	Ca	Na	K	Mg	Zn	Fe	Al	Li	Ni	Sr
LOQ	0.03	0.5	1.00	0.03	0.67	0.33	0.67	0.20	0.99	0.03
LOD	0.01	0.15	0.3	0.01	0.2	0.1	0.2	0.06	0.30	0.01

Data analysis

Statistical analysis of the data was performed using Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA). All data were expressed as mean and standard error mean. Differences in concentrations of the analyzed chemical elements between farms with different environmental burden were compared by the Scheffe test. A probability level of p<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The main goal of the presented study was to compare and monitor the concentration of essential and toxic elements in cow's milk, which comes from farms in areas with different environmental loads in Slovakia. Table 2 shows the average concentrations of 10 monitored elements in cow's milk on selected cattle farms.

Concentrations of monitored elements decreased in the following order: Ca>K>Na>Mg>Fe>Zn> Al>Li>Sr>Ni on farm with undisturbed environment, K>Ca>Na>Mg>Fe>Zn> Al>Sr>Li>Ni on farm with slightly disturbed environment and K>Ca>Na>Mg>Zn>Fe>Al> Li>Sr >Ni.

Table 2 – Mean concentrations essential and toxic elements in milk on selected farms (mg.kg-1)

	Farm in area with undisturbed environment "Zamagurie"	Farm in area with slightly disturbed environment "Poiplie - Novohrad"	Farm in area with strongly disturbed environment "Podunajsko"
	Mean±SE	Mean±SE	Mean
Ca	614.3964±33.754b	904.421±17.685a	916.033±14.666a
Na	161.417±14.335b	330.064±4.835a	313.050±5.484a
K	609.255±52.841b	1122.510±17.298a	1037.271±8.072a
Mg	54.554±3.539b	86.589±1.843a	84.641±1.344a
Zn	0.687±0.151b	0.100±0.001c	1.751±0.146a
Fe	1.449±0.091a	1.017±0.023b	1.506±0.1a
Al	0.100±0.001a	0.100±0.001a	0.813±0.672a
Li	0.023±0.004a	0.030±0.001a	0.030±0.001a
Ni	ND	ND	ND
Sr	0.145±0.01b	0.249±0.007a	0.005±0.001b

ND- not detected

Of the basic minerals, calcium is the most important element of milk that most people associate with bone health. Sufficient intake of calcium plays an important role in the development of children, giving bones strength and density, which is important in the prevention of osteoporosis (Bilandžić et al., 2019). The highest concentration of Ca (916.421 mg.kg-1) was recorded on a farm with a strong disturbed environment compared to the other farms. In our previous study, we found similar levels of Ca (981 mg.kg-1) on a farm from Spiš region (Ester Slovakia) during the spring breeding season, while calcium concentrations were higher during summer and autumn (1424 mg.kg-1 and 1214 mg.kg-1). In the region from an environmentally unstressed environment, calcium in milk samples was in a significantly higher concentration at the level of 1930 mg.kg-1 (Toman et al., 2020). In our next study, we found almost the same calcium concentration compared to the current study, where the calcium concentration was at the level of 915 mg.kg-1 in the spring period, but in the

autumn period, the calcium concentration increased to 1210 mg.kg-1 (Pšenková et al., 2016). The calcium values found in this study were higher than in China reported by Chen (2020), comparable to the results from Turkey (Bakircioglu et al., 2018), but lower compared to the calcium concentrations in Croatia reported in their study by Bilandžić, 2021.

Research suggests that a diet high in Ca, Mg and K and low in Na can prevent high blood pressure and cardiovascular disease (Bilandžić, 2021). A statistically significant difference in K concentration ($P<0.05$) was recorded in the case of the undisturbed area compared to the other monitored areas. Concentrations of K in this study were lower than concentrations in Croatia (Bilandžić, 2015), Turkey (Bakircioglu et al., 2018) and China (Chen et al., 2020). Capárová et al. (2009) reported that it is important to monitor milk K and Na content because hypokalemia represents an electrolyte imbalance that occurs in critically ill cattle. In the case of Na, we found a statistically significant difference ($P<0.05$) on the farm from the undisturbed

environment compared to the other monitored farms. Higher sodium concentrations in cow's milk were found by authors from Croatia (Bilandžić, 2015) and Pakistan (Kandhro et al., 2022), while countries such as Turkey (Bakircioglu et al., 2018) and China (Chen, 2020) reported lower sodium concentrations than in this study. In the case of magnesium, we also noted a statistically significant difference ($P < 0.05$) when comparing farms on a farm with an undisturbed environment, and almost the same concentration of magnesium was found on the other two farms. Higher values of magnesium compared to our detected concentrations were in Croatia (Bilandžić, 2015) and in China (Chen, 2020), on the contrary, lower compared to the reported results.

Iron is essential for maintaining proper cell function and is involved in the transport of respiratory gases (Lieu et al., 2001). Milk is not considered to be a good source of iron and its low concentrations do not indicate a sufficient need in humans, but its presence in milk has been shown to have a positive effect on inhibiting the occurrence of bacteria in milk (Bailey et al., 2011). Our results correspond to the occurrence of iron in milk, and thus its concentrations were also low in the samples we found and a statistically significant difference ($P < 0.05$) when comparing farms was noted in the case of a farm with a slightly disturbed environment. In our previous study, we recorded even lower concentrations of iron in milk originating from the region of eastern Slovakia compared to the present study (Toman et al., 2020). Iron concentrations in milk from Poland were lower (0.173 and 0.2 mg.kg⁻¹) than concentrations from all regions of this study (Górska a Oprzadek, 2006; Gorska and Oprzadek, 2011). Almost three times higher iron concentrations were found in Turkey compared to Fe concentrations in the regions of this study (Kaptan et al., 2016;

Bakircioglu et al., 2018). The recommended amount of daily iron intake for the adult population of the Slovak Republic without specific needs is an average of 10 mg for men and 15 mg for women (Ministry of Health of the Slovak Republic, 2015). It follows that milk is really not a sufficient source of iron to cover the daily allowance for this element.

Zinc acts as a catalytic structural and regulatory element and plays an important key role in cellular homeostasis (Wang et al., 2014). In a previous study in milk from eastern Slovakia, we recorded zinc concentrations from 3.44 to 4.8 mg.kg⁻¹, depending on the season (Toman et al., 2020). Compared to the presented results, we recorded a lower average annual concentration of zinc in all areas. And in all three cases, we noted statistically significant differences in the case of zinc ($P < 0.05$).

Aluminum is among the toxic elements that mainly damage the nervous system, skeleton and hematopoiesis (Ayar et al., 2009). In all monitored areas, we recorded very low concentrations of aluminum, in the case of the first two farms only a few tenths of a milligrams. However, Kaptan et al. (2016) found aluminum concentrations in raw cow's milk as high as 17.32-22.50 mg.kg⁻¹. By analyzing the non-carcinogenic risk of exposure to Al in the last decade in the world, Boudebbouz et al. (2021) concluded that the consumption of milk in terms of the amount of this element received is safe for humans. Our results are within the range of safe values analyzed by these authors.

Toxic elements such as Li and Sr were also detected in the analyzed milk samples, but in very low concentrations, and nickel was below the detection limit. Although in the case of Li, Ni, Sr these are little-studied elements in milk, their occurrence in the environment is in some cases very significant. The toxic effects of these metals are significant especially from long-term intake. They have a

negative effect on the kidneys, the nervous system, the cardiovascular system, the skin, the thyroid gland, the lungs, and in addition, they can have carcinogenic and mutagenic potential (Filtova and Cherpek, 2020; Peana et al., 2021). When comparing the farms in terms of the occurrence of monitored toxic elements between the farms, we did not notice any statistically significant difference.

Saribal (2020) states that Turkey is among the countries with significant sources of Sr, and concentrations ranging from 378.9-984.3 $\mu\text{g}\cdot\text{l}^{-1}$ have been found in supermarket cow's milk. These values slightly higher than in our study. Average concentrations of 0.43 $\text{mg}\cdot\text{kg}^{-1}$ were found in commercially available cow's milk in Italy, which were also higher compared to our results (Astolfi et al., 2020). Strontium is considered one of the possible causes of osteomalacia (Cohan-Solal, 2020; Ricci et al., 2021).

CONCLUSIONS

Differences in concentrations of essential and toxic elements in cow's milk are caused by many factors (geographical location, geological substratum, pasture, type of soil, animal nutrition, age, breed, lactation period). However, according to various authors, the environmental load also plays a role. From all monitored elements, concentrations of Ni were below the LOD limits. Mean concentrations of Al, Li and Sr was very low and differences in concentrations between areas was not statistically significant. From essential elements, the highest concentrations were found in case of Ca and Na and statistically significant differences ($P < 0.05$) was detected between undisturbed and slightly disturbed area of Slovakia in case of Ca, Na, K and Mg. We can conclude that milk from all monitored areas in this study is a good source of essential elements that are necessary for the optimal functioning of the human

body. The average concentrations of elements Ca, Mg and K were lower compared to other available sources, but within the regular consumption of milk and milk products they are an important part of the daily intake. Considering the importance and frequency of cow's milk processing, we can consider it safe from these areas, and determining the concentrations of elements in cow's milk plays an important role in evaluating the nutritional value and the state of the environment in the future as well.

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