RESEARCH PAPER

DETERMINATION OF SELECTED HEAVY METALS IN COW'S MILK FROM POLOG REGION

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Abstract
Milk and dairy products are very common in our nutrition, since it is a source of some vitamins and a lot of mineral constituents which are necessary for proper development and functioning of different tissues and organs. In this study a total of 105 milk samples was collected from cattle farms located in seven (7) different rural areas in Polog region: Bogovinje, Janciste, Jegunovce, Negotino, Poroj, Slatino and Toplica. Individual raw milk samples were taken from five (5) cows in lactation of each selected farm (seven farms in total) in the winter – spring season. A survey was conducted in three (3) repetitions. The concentration of heavy metals cadmium and nickel (Cd and Ni) and metalloid zinc (Zn) in raw milk has been determined by Graphite Atomic Absorption Spectroscopy of Agilent 240Z. The highest concentration of Cd was found in the samples of dairy farms from Bogovinje area (1.1163 μg/L). The concentration of Ni was highest in the samples of dairy farms from Janciste area (73.433 μg/L). What concerns to Zn concentration in milk samples, the highest was found in the Toplica area (3.666 μg/L). These values are compared with authorized limit by FAO/ WHO standards as well as with the corresponding values of different countries available in the literature.
Key words: *heavy metals, cow's milk, atomic absorption spectrometer, Polog region.*

Introduction

Throughout the world, more than six billions people use milk and dairy products as the primary source of nutrients in diets (Buldini et al., 2002). In the ash (dry matter) of cow's milk contain some main elements as well as calcium, phosphorus and magnesium..In addition to potassium, sodium and chlorine in cow`s milk are identified a wide range of trace elements, such as zinc, copper, iron, manganese and iodine (Van der Berg, J. C. T., 1988). The almost ubiquitous presence of some metal pollutants, especially cadmium, lead and nickel in the environment facilitates their entry into the food chain, thus increasing the hazard of human and animal health (Licata et al., 2004). In general, cow milk contains very low concentrations of heavy metals (Lopez et al., 2002), which, however, increase at times, though their excretion through the milk is proportionally very low (Miller, 1971). Accumulation of heavy metals in the ecosystem (water, soil, plant and animal) makes them very toxic and leads to undesirable consequences for live organisms (Bogut et al., 2000). According to Nwude et al. (2010), blood being a major medium of transfer of heavy metals into milk. Thus, milk can be

considered as a bio indicator of industrial pollution. The biggest pollutant in the Polog planning region is Jugohrom Ferroalloys DOO Jegunovce (previously a state owned company Silmak since 1952) which is located in the Macedonian village Jegunovce, in the Region of Polog valley. Jugohrom produces ferro-alloy, particularly ferro-silicon. The company has a history of over 50 years of uncontrolled disposal of waste material containing chromium salts caused soil and groundwater pollution, including the River Vardar, an important trans-boundary river. (The Law on Environmental Protection, 2005; The Third National Plan on Climate Change, 2013).

Identification and evaluation of the concentration levels of some hazardous metals in cow`s milk that are product in some farms in agricultural areas in Polog region such are Zn, Cd and Ni are the aims of present study. The objective is the quantitative assessment of the concentration of these hazardous metals in the raw cow`s milk.

Materials and methods

Collection of milk samples

Sampling: Raw milk samples were collected in winter - spring period (January - April), 2019 in some dairy farms, random selected, seven different areas in Polog region: Bogovinje, Janciste, Jegunovce, Negotino, Poroj, Slatino and Toplica. (Polog planning region is situated in the northwest part of Macedonia, with an area of 2,416 km^2).

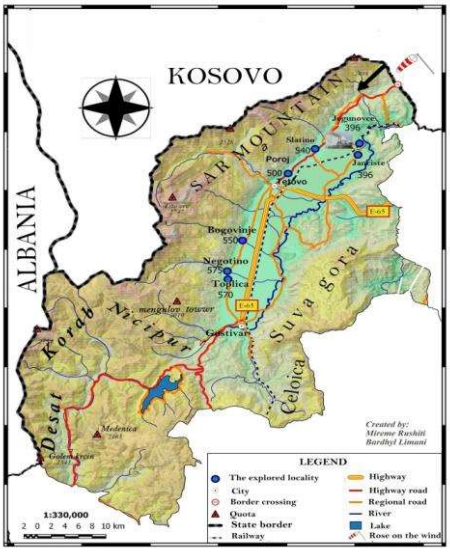

Fig. 1. Map of milk samples collection

Sample preparation and analyses of metals

A total of 105 milk samples was analyzed. The analysis of each milk samples was repeated three times for each element. For identification of Zn, Cd and Ni metals the AOAC (1990) methods were used. According this method the quantitative assessment of the heavy metals in milk was carried out following to phases: (a) digestion (combustion, decomposition) of the material, and (b) determination of the concentration of heavy metals in the digested material by means of atomic absorption spectroscopy. Milk was digested by applying the method of wet combustion according to **Soylak et al. (2004**), which included the following procedures:

• Measurement of 5ml fresh milk and transfer to Kjeldahl flasks of 100 ml;

• Decomposition / combustion of the material with organic combustion HNO3 / H2O2 (2:1) (12 ml for 5 ml sample); These flasks were then placed in a sandy bath and burnt at a temperature of (140 °C) for a time period of 12 hours. Then, the contents of the burnt material (1-2 ml) are filtered and transferred to the measured zucchini (25 ml) by adding distilled water and then transferred to the tubes. The resulting solutions from the burned material

were maintained in a refrigerator (4ºC) before being used to analyze the content of heavy metals. Three blind tests were prepared. The concentration of heavy metals in the solutions in the test tubes was determined using Graphite Atomic Absorption Spectroscopy of Agilent 240Z.

The content of heavy metals in the samples (γ) was expressed as mg • kg^{-1} or μg • kg^{-1} according to the following formula:

$$\gamma = \frac{c \cdot V - S}{m},$$

where:

c - the concentration of the solution read on AAS (ppm or ppb);

V -represents the volume of the solution of the combustible material (25 ml)

S - the concentration of the blank in the blank (average value of the three blind samples); *m* is the sample mass (0.1 - 0.5 g).

Statistical analysis – The main statistical parameters, like averages and variances, was evaluated using the methods of descriptive statistical analyze -Data Analyze ToolPak.

Results and discussion

Concentration of heavy metals in raw cow milk samples

The averages values of the elements, Zn, Ni and Cd in milk samples collected from different areas in Polog region are given in Table 1 and Figure 2.

The values of the concentration of Cd in milk ranged from 0.1460 to 1.1163 (μg/L) during whole sampling period (3 repetitions). The maximum level of Cd was observed on the dairy farm in Bogovinje and the minimum level was determined in samples from dairy farm in Toplica. The highest concentration of Ni was found in the samples of dairy farms from Janciste area (73.433 μg/L) and the lowest ones in the samples from Toplica area (29.923 μg/L). In raw milk samples the highest level of Zn was found in the dairy farm from Toplica (3.666 μg/L). Mean values for the elements Zn /Ni /Cd were determined as: 3.3252 / 43.2259 / 0.3575 (μg/L) which were less than the authorized limit by FAO /WHO standards.

Table 1.Mean values of Zn, Ni and Cd in raw milk samples collected in the random selected farms in different zone of the Polog region ($\bar{x}$ / SD)

Areas	Zn (μg/L)	Ni (μg/L)	Cd (μg/L)
Bogovinje	2.938	37.708	1.116
Janciste	3.638	73.433	0.262
Jegunovce	2.889	36.993	0.256
Negotino	3.311	27.373	0.181
Poroj	3.506	32.173	0.207
Slatino	3.335	63.873	0.466
Toplica	3.666	29.923	0.146
$\bar{x}$ / SD	3.32 ± 0.28	43.22±16.72	0.36±0.31

In all the samples, measured cadmium and nickel contents were less than the authorized limit by the FAO /WHO standard. There is a wide variation in the published data for the elemental concentrations of cow's milk of different countries (Ostapczuk P. et al., 1987; Baranovska I., 1994; Lopez A. et al., 1995).

It is well-known that the concentration on heavy metal heavy metals causes hazardous effects on human organs. Consequently, efforts and care to reduce their amount in the organism through the reduction of the level of their concentration in the foods we consume is a very important protection measure for human health. The results of the analyzes carried out in the framework of this study show that in milk produce by the cows breeding in the conditions of small scale family production

system in North Macedonia, the levels of concentration of the Zn, Ni and Cd are similar or lower than those reported earlier from other regions of the world (Licata et al. (2004). Rodrigues et al. (1999), have communicated that the concentration of heavy metals in samples of raw cow's milk in Spain, and the average concentration of cadmium was 4.88 µg/L, which was higher than cadmium levels in our study.

Conclusions

According to the presented data it can be concluded that the highest concentration of Cd was found in the samples of dairy farms from Bogovinje area (1.1163 µg/L). The concentration of Ni was highest in the samples of dairy farms from Janciste area (73.433 µg/L). What concerns to Zn concentration in milk samples, the highest was found in the Toplica area (3.666 µg/L). In all the samples, measured zinc, cadmium and nickel concentrations were less than the authorized limit by FAO /WHO standard. Further studies are necessary to evaluate the contents of other toxic metals on a greater number of milk samples in Polog region (more than 50 years polluted area) to confirm the absence of possible toxicological risks.

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