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Original paper

Concentration of Toxic Heavy Metals in Raw Milk from Dairy Farms in R. N. Macedonia

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Abstract

Background and aim: The presence of toxic heavy metals is a matter of importance due to their ability to contaminate and damage the trophic chain. Determination of the content of heavy metals in raw milk is an important indicator of the quality and safety of this product, and the level of pollution in the region where the milk is produced. The main purpose of this research was to examine the content and distribution of toxic elements (cadmium - Cd, lead - Pb, copper - Cu, zinc - Zn, nickel - Ni and manganese - Mn) in individual samples of cow's milk from seven different locations.

Materials and methods: A total of 140 samples of milk were taken. For the assessment of the elements an Atomic Absorption Spectrometry technique (Agilent Technologies 55) was used. Statistically, the results obtained from the conducted surveys were interpreted using variation - statistical methods, which are applied for scientific research purposes (ANOVA).

Results: In our results from the annual average value of the elements we found that the content of all elements except lead was within the normal prescribed permissible limits. The calculated annual average value of lead from all seasons is 35,937 µg / L or 0.035937 mg / L. The differences between the mean values for the content of lead in the milk, in the different seasons, were found at the level of 0.05.

Conclusion: This study will contribute to improving the awareness of the current state of contamination of milk with toxic elements in the country, as well as raising consciousness of the farmers and consumers about the importance of food safety and public health maintenance.

Keywords: *Toxic Heavy Metals, Milk, Dairy Farms, AAS*

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Introduction

Worldwide, more than six billion people use milk and dairy products as a major source of nutrients in the human diet. It is especially important in infant nutrition, as its protein and mineral composition is essential [1]. The mineral composition of milk includes several main elements such as calcium, phosphorus and magnesium, as well as potassium, sodium and chlorine and a wide range of trace elements, including zinc, copper, iron, manganese and iodine [2]. Therefore, the presence of high concentrations of heavy metals in dairy products can be a potential risk for serious diseases and public health problems [3] and for this reason the determination of heavy metal content in raw milk is an important indicator of the hygienic condition of this product, as well as the level of pollution in the region in which the milk is produced [4], [5]. According to some literature data, cow's milk contains very low concentrations of heavy metals [6], which, however, sometimes increase, although their excretion through milk is proportionally low [7]. Nwude et al. (2010) found that blood is the major medium for transporting heavy metals in milk, so that milk can certainly be considered as a bio-indicator of industrial pollution [8].

Selected heavy metals that are the aim of our study: lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), chromium (Cr) and nickel (Ni) are in principle potentially toxic elements if present in food in concentrations that are above the allowed limits and are also prescribed by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), as well as the legal norms of the Macedonian standards [9].

Through the obtained results, conclusions and recommendations for the public about the state in the country, the environmental awareness of the population will be strengthened as well as the increase of the awareness for consuming safe and quality products.

Material and Methods

As a working material in this doctoral dissertation is raw cow's milk produced in seven (7) selected farms, dispersed in seven different locations located at an appropriate distance from each other (7-10 km), selected according to the “wind rose” in the Polog region. The size of the farms is up to 30 heads, dairy cows are reared in tie -stalls. According to data provided by farmers, the average daily milk yield in all locations ranged from 18-25 liters per head.

Individual milk samples were taken in sterile plastic containers with caps (500 ml) by direct manual milking of the udder, according to a random selection of cows. The samples were taken seasonally, which means that a total of 140 milk samples (35 x 4) were analyzed from all farms. For the examination of the elements in the milk, five (5) individual samples were taken from each farm, or a total of 35 samples from seven farms (7x5). A total of six (6) heavy metals were examined (cadmium - Cd, lead - Pb, copper - Cu, zinc -Zn, nickel - Ni, manganese - Mn). The manner of sampling and transport was performed in accordance with the Rulebook on storage of raw milk and sampling for analysis and super analysis [10].

An Atomic Absorption Spectrometry technique (Agilent Technologies 55) with deuterium background corrector was used to analyze heavy metals in milk. The elements Pb, Cd, and Ni were determined by HGA graphite furnace using high purity argon. The other measurments were performed with a flame air / acetylene technique. The preparation of samples and determination of the concentration of heavy metals in milk consisted of two laboratory phases:

1) digestion (combustion, decomposition) of the material and

2) determination of the concentration of heavy metals in the digested material by means of atomic absorption spectrometry. Milk was digested using the wet combustion method according to Soylak et al. (2004) [11].

Statistically, the obtained results were interpreted using variation - statistical methods, which are applied for scientific research purposes (ANOVA).

Results

The annual mean values of heavy metals according to the obtained average values by seasons from all locations are shown in Table 1.

Table 1. Annual average value of heavy metals according to the obtained values by seasons from all locations (Cu, Mn, Zn - mg / L) and (Cd, Pb, Ni - µg / L)

Elements	Winter	Spring	Summer	Fall	$\bar{x}$
Cu	0.093	0.137	0.151	0.132	**0.128**
Cd	0.351	0.078	0.125	0.176	**0.183**
Pb	61.854	9.418	28.689	43.787	**35.937**
Mn	0.156	0.072	0.129	0.036	**0.098**
Ni	33.429	72.350	40.138	10.776	**39.173**
Zn	3.347	2.953	3.229	3.604	**3.283**

The lowest mean value of copper obtained during the analysis of milk samples from all seven locations by seasons was determined in the winter season and was 0.093 mg / L, while the highest mean was registered in the summer season 0.151 mg / L. The calculated annual mean value of copper from all seasons is 0.128 mg / L. During the analysis of milk samples from the selected locations by seasons, the lowest mean for cadmium was determined in the spring season and was 0.078 µg / L / 0.000078 mg / L, while the highest mean value was registered in the winter season 0.351 µg / L / 0.000351 mg / L. The annual mean value of cadmium calculated from all seasons was 0.183 µg / L / 0.000183 mg / L. From the data in Table 1 for lead concentration, the lowest mean value of the seasons was registered in the spring season 9.418 µg / L / 0.009418 mg / L, while the highest mean was determined in the winter season and was 61,854 µg / L / 0.061854 mg / L. The calculated annual mean of lead from all seasons is 35,937 µg / L /0.035937 mg / L. The lowest mean of manganese obtained during the analysis of milk samples by seasons was determined in the fall season and was 0.036 mg / L, while the highest mean value was determined in the winter season 0.156 mg / L. The annual mean of manganese from all seasons was 0.098 mg / L. From the results obtained from the analysis of milk from the selected locations by seasons, the lowest mean for nickel was determined in the spring season and was 72,350 µg / L / 0.07235 mg / L, while the highest mean value was registered in the fall season 10,776 µg / L / 0.010776 mg / L. The annual mean value of nickel calculated from all seasons was 39,173 µg / L / 0.039173 mg / L. For zinc content, the lowest mean per season was determined in spring and was 2,953 mg / L, while the highest mean value was registered in fall season 3,604 mg / L. Otherwise, the annual mean value of zinc from all seasons was 3,283 mg / L.

Table 2. Comparison between seasons for heavy metal content in milk samples from all locations
Multiple Comparisons (Tukey HSD)

Dependent Variable	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cu	winter	spriing	-.04371	.02524	.311	-.1094	.0219
		summer	-.05771	.02524	.106	-.1234	.0079
		autumn	-.03914	.02524	.410	-.1048	.0265
	spriing	winter	.04371	.02524	.311	-.0219	.1094
		summer	-.01400	.02524	.945	-.0796	.0516
		autumn	.00457	.02524	.998	-.0611	.0702
	summer	winter	.05771	.02524	.106	-.0079	.1234
		spriing	.01400	.02524	.945	-.0516	.0796
		autumn	.01857	.02524	.882	-.0471	.0842
	autumn	winter	.03914	.02524	.410	-.0265	.1048
		spriing	-.00457	.02524	.998	-.0702	.0611
		summer	-.01857	.02524	.882	-.0842	.0471
Cd	winter	spriing	.27114*	.08091	.006	.0607	.4816
		summer	.22629*	.08091	.030	.0158	.4368
		autumn	.17486	.08091	.140	-.0356	.3853
	spriing	winter	-.27114*	.08091	.006	-.4816	-.0607
		summer	-.04486	.08091	.945	-.2553	.1656
		autumn	-.09629	.08091	.634	-.3068	.1142
	summer	winter	-.22629*	.08091	.030	-.4368	-.0158
		spriing	.04486	.08091	.945	-.1656	.2553
		autumn	-.05143	.08091	.920	-.2619	.1590
	autumn	winter	-.17486	.08091	.140	-.3853	.0356
		spriing	.09629	.08091	.634	-.1142	.3068
		summer	.05143	.08091	.920	-.1590	.2619
Pb	winter	spriing	52.86771*	12.00533	.000	21.6409	84.0945
		summer	33.16571*	12.00533	.033	1.9389	64.3925
		autumn	18.06743	12.00533	.437	-13.1594	49.2943
	spriing	winter	-52.86771*	12.00533	.000	-84.0945	-21.6409
		summer	-19.70200	12.00533	.359	-50.9288	11.5248
		autumn	-34.80029*	12.00533	.022	-66.0271	-3.5735
	summer	winter	-33.16571*	12.00533	.033	-64.3925	-1.9389
		spriing	19.70200	12.00533	.359	-11.5248	50.9288
		autumn	-15.09829	12.00533	.591	-46.3251	16.1285
	autumn	winter	-18.06743	12.00533	.437	-49.2943	13.1594
		spriing	34.80029*	12.00533	.022	3.5735	66.0271
		summer	15.09829	12.00533	.591	-16.1285	46.3251

Mn	winter	spriing	.08371*	.01793	.000	.0371	.1304
		summer	.02686	.01793	.442	-.0198	.0735
		autumn	.12000*	.01793	.000	.0734	.1666
	spriing	winter	-.08371*	.01793	.000	-.1304	-.0371
		summer	-.05686*	.01793	.010	-.1035	-.0102
		autumn	.03629	.01793	.184	-.0104	.0829
	summer	winter	-.02686	.01793	.442	-.0735	.0198
		spriing	.05686*	.01793	.010	.0102	.1035
		autumn	.09314*	.01793	.000	.0465	.1398
	autumn	winter	-.12000*	.01793	.000	-.1666	-.0734
		spriing	-.03629	.01793	.184	-.0829	.0104
		summer	-.09314*	.01793	.000	-.1398	-.0465
Ni	winter	spriing	-34.56429*	6.11750	.000	-50.4764	-18.6522
		summer	-6.70914	6.11750	.692	-22.6213	9.2030
		autumn	22.65229*	6.11750	.002	6.7402	38.5644
	spriing	winter	34.56429*	6.11750	.000	18.6522	50.4764
		summer	27.85514*	6.11750	.000	11.9430	43.7673
		autumn	57.21657*	6.11750	.000	41.3045	73.1287
	summer	winter	6.70914	6.11750	.692	-9.2030	22.6213
		spriing	-27.85514*	6.11750	.000	-43.7673	-11.9430
		autumn	29.36143*	6.11750	.000	13.4493	45.2735
	autumn	winter	-22.65229*	6.11750	.002	-38.5644	-6.7402
		spriing	-57.21657*	6.11750	.000	-73.1287	-41.3045
		summer	-29.36143*	6.11750	.000	-45.2735	-13.4493
Zn	winter	spriing	.39400	.19370	.181	-.1098	.8978
		summer	.11886	.19370	.928	-.3850	.6227
		autumn	-.25657	.19370	.549	-.7604	.2472
	spriing	winter	-.39400	.19370	.181	-.8978	.1098
		summer	-.27514	.19370	.489	-.7790	.2287
		autumn	-.65057*	.19370	.006	-1.1544	-.1468
	summer	winter	-.11886	.19370	.928	-.6227	.3850
		spriing	.27514	.19370	.489	-.2287	.7790
		autumn	-.37543	.19370	.217	-.8792	.1284
	autumn	winter	.25657	.19370	.549	-.2472	.7604
		spriing	.65057*	.19370	.006	.1468	1.1544
		summer	.37543	.19370	.217	-.1284	.8792

* The mean difference is significant at the 0.05 level.

Anova statistics for examining the differences between the mean values of the elements in different seasons are shown in Table 2. There is no significant difference in the copper content of milk between the mean values in different seasons. Significant differences in the mean values of cadmium in milk content were found at the level of 0.05. The differences between the mean values are significant in the seasons spring - winter (0.27114*) and summer - winter (0.22629*). For the content of lead in milk, significant differences were found in the mean values at the level of 0.05. The differences are significant in the seasons spring-winter (52.86771*), summer-winter (33.16571*) and autumn-spring (34.80029*). The differences between the mean values of manganese in milk show a significance level of 0.05. There are significant differences between the seasons spring - winter (34.56429*), autumn - winter (22.65229*), summer - spring (27.85514*), autumn - spring (57. 21657*), autumn - summer (29.36143*). For the content of nickel in milk, significant differences were found in the mean values at the level of 0.05. The differences are significant in the seasons spring-winter (34.56429*), autumn-winter (22.65229*), summer-spring (27.85514*), autumn-spring (55.21657*) and autumn-summer (29.36143*). Significant differences in the mean values of zinc content in milk were found at the level of 0.05. The differences between the mean values are significant in the spring-autumn seasons (0.65057*).

Discussion

In the survey conducted by Malhat et al. (2012) [12] the highest average concentration is that of zinc 10.75 µg / g = mg / L and lead 4,404 µg / g = mg / L, while the lowest average concentrations are 2,836 and 0.288 µg / g = mg / L for copper and cadmium, respectively. Therefore, the results showed that most of the milk samples from different locations contained all elements at concentrations higher than those recommended for milk according to FAO International Standards and the Code. Górska et al. (2007) [13] found that the concentration of lead in cow's milk was 0.003 mg / kg. In other European countries, the Pb content in milk (mg / kg) was as follows: Slovenia - 0.05 and Spain - 0.0018 [14] and Romania - from 0.052 to 0.617 mg / kg [15]. In previous studies conducted by Limani et al. (2019) [16] in the Polog region, the average value of lead in the period January - May was 278.21 µg / L / 0.27821 mg / L. Otherwise, in research done by Anastasio et al. (2006) [17] also reported increased levels of cadmium and lead in samples of milk originating in Italy. Zinc concentrations in our milk samples were lower than those recorded in cow's milk from Northern Spain [18] and those determined from Turkey [19], as well as those in cow's milk. milk from Silesia, Poland [20] and Pakistan [21]. Also in our study, Cu concentrations were lower than those measured in cow's milk from Turkey [20], as well as Cu concentrations in cow's milk from Croatia and Pakistan. [22], [21]. On the other hand, the Mn values in cow's milk were lower than the concentrations determined by authors from South Africa [23]. In the study by Tizhoosh M., & Tizhoosh HR., (2016) [24] average concentrations of heavy metals in raw milk produced by dairy industry in Koramabad, Iran, expressed in (mg / L) were: 3.07 (Zn), 2.72 (Pb), 0.1 (Cd) and 0.14 (Cu). The results obtained from the comparison of the average concentrations measured by the WHO guidelines show a statistically significant difference ($p < 0.05$) which indicates that the average concentration of each element in the studied sample is higher than the WHO recommendations, mainly due to the release of heavy metals through animal feed. and drinking water.

Conclusion

In our research the annual average value of the elements we found that the content of all elements except lead was within the normal prescribed permissible limits.The highest lead content was recorded in the samples in the winter season 61,854 µg / L / 0.061854 mg / L, and the calculated annual mean value of lead from all seasons was 35,937 µg / L or 0.035937 mg / L. The differences are significant in the seasons spring-winter (52.86771*), summer-winter (33.16571*) and autumn-spring (34.80029*). The differences between the mean values for the content of lead in the milk, in the different seasons, were found at the level of 0.05. This study will contribute to improving the awareness of the current state of contamination of milk with toxic elements in the country, as well as raising the consciousness of the farmers and consumers about the importance of food safety and public health maintenance.

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Conflict of interests
The authors declare no conflict of interests regarding the publication of this paper.

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