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Assessment of Heavy metals Concentrations in Cow's Milk collected from Polog Region, Republic North Macedonia

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Abstract: *Milk and dairy products are one of the most important and prominent products, part of human nutrition, since it is a source of numerous essential nutrients needed for the development and functioning of the organism. The objective of this study was to determine the concentration of heavy metals in raw cow's milk samples from different rural areas in Polog region: Jegunovce, Janciste, Bogovinje, Negotino, Poroj, Slatino and Toplica. In the period from January to May 2019 a total of 105 milk samples were collected from seven (7) dairy farms. Individual raw milk samples were taken from five (5) cows in lactation of each selected farm. A survey was conducted in three (3) repetitions. Selected metals as: Cu, Mn, Cd, Pb, Ni and metalloid (Zn) in raw milk samples have been assessed by Atomic Absorption Spectroscopy of Agilent 240 Z. Determined values were compared with the authorized limit by FAO/ WHO standards as well as with the corresponding values of different countries available in the literature.*

Index Terms: *Atomic Absorption Spectrometer, cow's milk, heavy metals, Polog region*

1. INTRODUCTION

The modern human, uncontrolled population growth, technological progress, various industrial activities and increased roadway traffic have caused a significant increase the toxic heavy metals in the environment and affected all the global air, water and food **[5]**.Heavy metals are considered to be cumulative poisons, so that poisoning is a consequence of chronic exposure, over long periods and repeated small doses, leading to bioaccumulation to the toxic level **[8].** Due to the growing environmental pollution, it is necessary to determine and monitor the levels of heavy metals in milk, because they can significantly influence human health **[4],** many reports indicates the presence of heavy metals in milk and other food products **[12,14]**. There are about 38 micro and trace elements reported to be found in raw milk from various regions around the world **[1]**. These mineral content in raw cow's milk may vary depending on several non- genetic factors, i.e. lactation period of cows, annual feed composition, seasonal variations and climatic conditions, as well environmental pollution **[4,16]**. However, at high concentrations, even essential metals cause toxicity and it is closely related to age, sex, daily intake, duration of exposure, absorption rate and efficiency of excretion **[11,15].** In R. North Macedonia, the situation is no better by the activities of most industries and the populace towards waste disposal and management, which usually leads to the increasing level of pollution of the environment. The biggest pollutant in the Polog planning region is Jugohrom Ferroalloys DOO Jegunovce (previously a state owned company Silmak from 1952), located in the Macedonian village Jegunovce, in the

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Region of Polog valley. Jugohrom produces Ferro-alloy, particularly Ferro-silicon and is the main employer in Jegunovce. 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 **[18]**. The main purpose of the current study therefore is to determine the concentration of selected hazardous metals (Cu, Mn, Cd, Pb, Ni and metalloid Zn) in cow's milk samples from dairy farms in Polog region, and to compare these obtained values with the literature data and, based on that, to draw conclusions on the safety of milk in human nutrition.

2. MATERIALS AND METHODS

2.1. Description of Study Area and milk sampling

The study was conducted in Polog region, located in the northwestern part of Republic North Macedonia, with an area of 2.416 km2 and is about 55 km long and 8-10 km wide, surrounded by the mountains: Shara, Suva Gora and Zedin.

Geologically, it is a typical tectonic valley that is deeply diminished Paleozoic mass of various rocky formations except at the north-eastern edge where the Mesozoic ultrabasic rocks are represented in the Paleolithic. The north wind occurs at high frequencies in all months of the year with an average annual velocity of 1.5 m/s and the maximum annual speed reaches 27 m/s.

Aside from the biggest industrial pollutant Jugohrom Ferroalloys DOO Jegunovce, in the Polog region, there are about 30 mini factories for furniture production, as well as a brick production plant - blocks that simultaneously contribute

to environmental pollution. Do not omit the pollution coming from the high frequency traffic on the regional road "Tetovo – Jazhince" (localities of Poroj and Slatino), as well as the paved highway "Mother Teresa" (localities Bogovinje, Negotino and Toplica).

Fig. 1. Map of the localities for milk samples collection

Raw milk samples were collected in the winter –spring season, (January to May 2019), from dairy farms located in seven (7) different areas in Polog region.

2.2. Sample preparation and analyses

A total of 105 milk samples at the amount of 500 ml from one cow were taken and analyzed in the present study. Each sample was repeated three times for each element. Physico - chemical parameters of milk samples expressed in % (fat, SNF, proteins, lactose, ash, freezing point, density) and pH were examined in the milk "Eko Shar", Tetovo with Laktoskan, model Funke Gerber, Type: Lacto Star Laboratortechnik 12105 Berlin, AppNr3510-170610. For determination Zn, Cd and Ni metals were used according to the AOAC (1990) methods **[2].** Determination of the content of heavy metals in milk covers 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 spectroscopy. Milk was digested by applying the method of wet combustion according to **[12],** which included the following procedures: measurement of 5ml raw milk and transfer to Kjeldahl flasks of 100 ml and decomposition/combustion of the material with organic combustion HNO3 / H2O2 (2:1) (12 ml to 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 marrows (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* is 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* is the concentration of the element in the blank (average value of the three blind samples); *m* is the sample mass (0.1 - 0.5 g).
Statistical analyses – mean values of three replicates were taken for each determination and were subjected to Excel Descriptive Statistic using Data Analyze ToolPak. The data was analyzed by t-test to examine the statistical significance of differences in the mean concentrations of the heavy metal composition of the different milk samples studied.



Pics. 1,2,3 and 4. Farm sampling and laboratory assessment of milk samples in Polog region

RESULTS AND DISCUSSION

In this study the precision of the results was evaluated by mean concentration of the triplicate samples (n=3), analyzed under the same conditions. The average physico-chemical composition and heavy metals concentrations in the samples of raw cow's milk from the seven (7) locations Jegunovce (J), Janciste (Ja), Bogovinje (B), Negotino (N), Poroj (P), Slatino (S), and Toplica (T) are given in Table 1 and Table 2. In Table 1 there are presented some composition characteristics of the raw milk samples from the dairy farms in Polog region.

Table 1. Composition characteristics of the collected milk from seven different locations

Parameters	**J**	**Ja**	**S**	**P**	**N**	**B**	**T**
($\bar{x}$)			**n=3**				
Fats %	4.940	3.490	4.226	4.436	2.962	3.412	2.034
Solids-non -fat %	9.160	8.320	9.038	9.024	8.768	9.882	9.058
Proteins %	3.440	3.114	3.400	3.384	3.444	3.732	3.412
Lactose %	4.710	4.278	4.662	4.640	4.724	5.114	4.686
Ash %	0.600	0.676	0.632	0.630	0.724	0.660	0.704
Viscosity %	1.027	1.025	1.027	1.027	1.029	1.032	1.030
Freezing point	-0.499	-0.479	-0.495	-0.497	-0.516	-0.117	-0.500
pH	6.76	6.72	6.68	6.76	6.74	6.78	6.80

Table 2. Mean concentration of selected elements in cow's milk samples

Elements (μg/L)	J	Ja	S	P	N	B	T
($\overline{x}$)				n = 3			
Cu	100 a	110 ab	150 abc	168 abcd	103 de	128 abcde	126 abcde
Mn	190 a	90 ab	200 abc	290 abcd	70 abcde	110 abcdef	90 cdef
Cd	0.256 a	0.262 ab	0.466 abc	0.207 abcd	0.181 abcde	1.116 abcdef	0.146 abcdef
Pb	27	73	33	40	42	54	50.52

	8.21a	.10ab	.15abc	.68abcd	.66abcde	.27abcdef	abcdef
Ni	36.993a	73.4333ab	63.8733abc	32.1733abcd	27.3733abcde	37.7083abcdef	29.923abcdef
Zn	2.8889a	3.6338ab	3.3335abc	3.5006abcd	3.3111abcde	2.9388abcdef	3.666abcdef

*[abcdef] The differences in the values with the same superscripts in the same raw are statistically significant at the level of p<0.5

WHO limits for Cu=2 mg/L; Mn= 0.05 mg/L; Cd = 0.01 mg/L; Pb = 0.01 mg/L; Ni = 0.05 mg/L and Zn = 2 mg/L The raw milk samples for heavy metals determination were analysed in triplicate, and the mean concentrations are found in Table 2. Average cooper (Cu) concentrations obtained ranges between 103 -168 (μg/L). The highest average level was registered in milk samples from P-locality (168 μg/L) and the lowest in samples from J – locality (100 μg/L). In their investigation carried out in R. Serbia, the authors noted content of copper in the tested milk samples which ranged from 0.100-0.401 mg/L **[17].** The measured values are in the range of values that, as adequate, gives **[9].** Average manganese (Mn) concentrations extended from 70 μg/L from N – locality and 260 μg/L from P- locality. The average values of the concentration of cadmium (Cd) in milk ranged from 0.146 to 1.116 (μg/L) from 3 repetitions. The maximum average value of Cd was observed from the dairy farm in the B-locality and the minimum level was determined in samples from the T-locality. Also, milk from all investigated farms, based on the content of Cd, c**orresponded to the requirements of the Regulation (2002) [6],**

which prescribes the maximum allowed content of Cd (max 0.01 mg/kg).
Data of our study are largely consistent with the data used by the authors from different countries. According to the authors **[10]**, the average concentration of cadmium in samples of raw cow's milk analysed in Spain, was 4.88 μg/L, (0.7–23.1 μg/L) which was higher than cadmium levels in our study.
The average lead (Pb) values ranged from 33.15 μg/L from S-locality to 278.21 μg/L in J –locality (according to the Regulations from Official Gazette of the Republic of Macedonia No. 54/2002, maximum permissible concentration for lead in raw milk is 0.02 mg/kg) **[6].** What regards to the investigation about exposition of lactating cows of lead and cadmium, the authors state that the content of Pb in cow's milk from unpolluted areas was 0.25 ± 0.03 μg/ml (0.00-0.79) **[7]**. Significant values for the content of Pb in milk are given by **[13].** The authors state that the average value of Pb content in cow milk from the industrial settlements in Romania amounted to 0.32 ± 0.12 mg/kg and concluded that it significantly exceeded the maximum allowable value. In our study the levels of lead in milk samples collected from all localities, especially from the J- locality was higher than the Regulatory limits and pose a health risk. To avoid impacts on collective health, the elaboration of a monitoring program for the quality of milk produced in the studied area, regarding the contamination by lead, is necessary, particularly for localities J and Ja. The highest concentration of Ni was found in the samples of dairy farms from J -locality (73.433 μg/L) which is above the WHO limit **[3]** and the lowest ones in the samples from T-locality (29.923 μg/L). In raw milk samples the highest level of Zn was found in the dairy farm from T- locality (3.666 μg/L). The content of Zn in cow's milk from dairy farms located in the smelter based on lead and zinc was 12.50 ± 0.73 μg / ml (7.96-15.95) **[7].** In this study among the individual farms there were no significant differences in the content of Zn in raw milk.

CONCLUSION

The present investigation on determination of selected elements in raw milk samples from seven different localities was preliminary work showing some trends in the impact of the environmental pollution on the heavy metals composition of cow's milk. According to the presented data the highest concentration of lead (Pb) which was above the limits was found in the samples of dairy farms from J -locality (278.21 μg/L). The concentration of Ni was highest in the samples of dairy farms from Janciste area (73.433 μg/L). In all the samples, measured cuprum, cadmium and zink concentrations were less than the authorized limit by the FAO /WHO standard. There was a significant difference in the concentrations of all elements among the milk samples of all seven localities at $p < 0.5$. Further studies are necessary to evaluate the contents of toxic heavy metals on a greater number of milk samples from various producers in Polog region (more than 50 years polluted area) and other regions in the Republic and to confirm the absence of possible toxicological risks.

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