

## CONTENT OF MAJOR AND TRACE ELEMENTS IN RAW EWES' MILK USED FOR PRODUCTION OF TRADITIONAL WHITE BRINED CHEESE

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### ABSTRACT

The content of minerals (major and trace elements) in raw ewes' milk produced in traditional way in different regions in Macedonia is the subject of this study. The households from where the milk samples were collected are exposed to different levels of anthropogenic pressure. The concentration of 17 elements (Ag, Al, As, Ba, Ca, Cd, Co, Cu, Fe, Mg, Mn, Na, Ni, P, Pb, Sr, and Zn) was analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) after performing microwave digestion. The analyses of ewes' milk did not show any significant variation in the levels of major elements. The concentration of Ca, Mg, Na and P were in the range of 1131-2070 mg.kg<sup>-1</sup>, 98.3-183 mg.kg<sup>-1</sup>, 223-400 mg.kg<sup>-1</sup> and 569-1080 mg.kg<sup>-1</sup>, respectively. The levels of Ag, As, Cd, Co, Ni and Pb in all the analyzed milk samples were below the detection limit although some households were located in areas exposed to environmental contamination with heavy metals (Cd, Pb and Zn). The raw ewes' milk contains Cu in the range of 0.66-1.47 mg.kg<sup>-1</sup>, Fe of 1.42-3.82 mg.kg<sup>-1</sup>, Mn of 0.04-0.16 mg.kg<sup>-1</sup> and Zn of 2.90-6.27 mg.kg<sup>-1</sup>. The soil composition, the traditional utensils and containers used for milk storage correlated with higher concentration of trace elements (Al, Ba, Cu, Fe, Mn, Ni, Sr, Zn) in some of the analyzed milk samples. The obtained results point out that ewes' milk produced in households and used for manufacturing of traditional dairy products is safe for consumption.

**Key words:** mineral elements; trace elements; ewes' milk

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### INTRODUCTION

Milk and dairy products are important sources of nutrients because they are rich in proteins, fats, hydrocarbons, vitamins and minerals. Minerals are also important for the human diet because of their structural, biochemical and nutritional functions (Zamaberlin *et al.*, 2012). All essential minerals can be found in milk and dairy products where major elements (Na, K, Ca, Mg, Cl and P) are present with higher concentration than the trace elements (Fe, Cu, Zn, Mn, Cr, Se). The mineral composition of milk depends on the genetic characteristics, stage of lactation, environmental

conditions, type of pasture, soil contamination and the health conditions of the animals (Borys *et al.*, 2006; Park *et al.*, 2007; Gonzalez-Martin *et al.*, 2009; Aly *et al.*, 2010).

The presence of major and trace elements in milk can indicate whether the product meets the needs of particular elements in human nutrition and may also indicate possible environment contamination (Borys *et al.*, 2006).

High concentration of trace elements (as well as heavy metals) in milk and cheese is the result of their growing concentration in the environment due to increase of urban, agricultural and industrial emissions (Caggiano

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*et al.*, 2005). Animals intake heavy metals through food chain, air and water. It is considered that ingestion of contaminated fodder is the main source of metal residue in the secreted milk (Elbarbary and Hamouda, 2013). The increasing industrialization and environmental pollution with heavy metals and their negative impact on human health (Perween, 2015) has raised the public interest in metal contamination of food stuffs. The processing equipment, reagents and accidental contamination during storage may also affect the dairy products (Elbarbary and Hamouda, 2013). That is why recently a lot of studies have been carried out on mineral and heavy metal content in milk and cheese (Dobrzański *et al.*, 2005; Anastasio *et al.*, 2006; Vural *et al.*, 2008; Aly *et al.*, 2010; Kodrik *et al.*, 2011; Lukáčová *et al.*, 2012) and their variation during ripening and seasonal changes (Cichoski *et al.*, 2002; González-Martín *et al.*; 2009; Kirdar *et al.*, 2013).

Unprocessed ewes' milk is a basic raw material for production of white brined cheese and other dairy products in traditional way in almost all regions in Macedonia, particularly the mountain areas. However, there are few studies regarding the mineral elements and heavy metals content in milk. Ivanova *et al.* (2011) analyzed the mineral content of milk from dairy sheep breeds. The mineral content of milk from three types of dairy sheep populations were compared, namely: the Bulgarian Dairy Synthetic population bred in Bulgarian, East-Friesian and the Awassi breeds bred in Macedonia. The results show a slight variation in the content of Ca and P among the studied breeds, and also that the content of trace elements in the milk is lower than the maximum permissible levels for the raw ewes' milk.

The aim of this study is to assess the concentration of mineral and trace elements in ewes' milk used for production of traditional dairy products, collected from different regions in Macedonia. It is also important to assess whether the trace elements in the collected milk samples did not exceed the allowed amounts as the households were located in areas exposed to different levels of anthropogenic pressure.

## MATERIALS AND METHODS

### Sampling sites

The study was conducted during May 2014 and the samples were collected from 12 households located in different regions in Macedonia exposed to different anthropogenic pressure. Three households (in the eastern region) were located near the "Sasa" lead and zinc mine, two households (in the central region) near the abandoned lead and zinc smelter in Veles and one household (near Skopje) near the oil refinery. The households from Sveti Nikole (eastern region), Bistra Mountain (western

region) and Mariovo (south-western region) were located in areas without any intensive pollution.

The sheep flocks included mainly Merinolandschaft crossbreeds. During the whole year the flocks fed on pastures with the exception of winter periods when the flocks fed with grazing and hand feeding (with hay and barley or concentrates).

### Sample collection

Total of 24 samples were taken from raw bulk milk collected in traditional vessels or plastic containers. Two milk samples from each household were taken in plastic laboratory bottles and brought to the laboratory in refrigerated condition.  $K_2Cr_2O_7$  was added to the milk samples in order to prevent curdling. Until the analyses were performed the samples were kept frozen (-18 °C).

### Sample analyses

The milk samples were analyzed for content of major elements (Ca, Mg, Na and P) and trace elements (Ag, Al, Ba, Cu, Fe, Mn, Sr, Zn, Ni, As, Pb, Cd, Co).

A microwave system was used for acid digestion of all of the samples. The milk samples were first dried at 70 °C and then grated. To each dried sample (0.5 g), 7 ml  $HNO_3$  (trace pure) and 2 ml  $H_2O_2$  were added. Then the same were left overnight to react and burn the organic material. The vessels with the material were then put in microwave digestion system (Mars<sub>x</sub>, CEM) for total digestion (15 min at 180 °C). After the digestion was complete the samples were filtered and poured in 25 ml bottles. After that the samples were measured. The concentration of minerals was expressed in  $mg \cdot kg^{-1}$  wet weight.

The content of elements in samples was determined using inductively coupled plasma - atomic emission spectrometry ICP-AES, (715-ES, Varian, USA) applying ultrasonic nebulizer CETAC (ICP/U-5000AT<sup>+</sup>) for better sensitivity.

Statistical analyses were conducted using F-test between the results gained from milk samples. The analyses were performed using the software package Statgraph 3.0 (Statistical Graphics, Warrenton, Virginia, USA).

## RESULTS AND DISCUSSION

The results obtained from the determination of major elements (Ca, Mg, Na and P) in milk are shown in Table 1. The concentration of K in milk was not analyzed due to  $K_2Cr_2O_7$  utilization for milk curdling prevention. The results show variations in the content of major elements in raw milk samples collected from different regions, but they are not significant ( $p < 0.05$ ). Regarding Ca concentration, the obtained results were

**Table 1: Content of major minerals in ewes' milk (mg.kg<sup>-1</sup> wet weight)**

Region	Household	Ca	Mg	Na	P
Sveti Nikole	1	1236	98.3	223	569
	2	1645	129	335	754
	3	1579	130	241	781
	Av ± Sd	1487 ± 219	119.0 ± 18.0	266 ± 60	701 ± 115
Sasa	4	1131	108	236	576
	5	1885	174	339	1051
	6	1444	132	241	738
	Av ± Sd	1487 ± 379	138.3 ± 34	272 ± 58	788 ± 242
Veles	7	2070	183	400	1080
	8	1508	134	252	721
	Av ± Sd	1789 ± 861	158.7 ± 76	326 ± 172	901 ± 421
Mariovo	9	1558	126	281	774
	10	1314	115	284	643
	Av ± Sd	1436 ± 354	120.5 ± 26	282 ± 64	709 ± 179
Skopje	11	1300	103	282	618
Bistra	12	1295	129	275	606

Average ± standard deviation

similar to the study of Ivanova *et al.* (2011). In view of Ca and P concentration, the levels were lower compared to the results of Polychroniadou and Vafopoulou (1985); Şahan *et al.* (2005); Boris *et al.* (2006); Ivanova *et al.* (2011) and Yabrir *et al.* (2014).

Variations were also observed regarding the presence of Mg and Na. The values obtained for Mg in this study were similar to the tests conducted by Şahan *et al.* (2005), but were lower compared to the results of Moreno-Rojas *et al.* (1993); Şahan *et al.* (2005); Boris *et al.* (2006); Güler (2007) and Yabrir *et al.* (2014). The lower values of major minerals in this study were probably affected by the manner of breeding and feeding the sheep, which throughout the year mainly feed on pasture vegetation, and barley, silage or concentrate are occasionally introduced in their nutrition. A diet with pasture vegetation means both unbalanced and lower minerals intake which later affects the mineral content of the milk (Ivanova *et al.*, 2011; Ahmad *et al.*, 2013). Physiological changes in the mammary gland, as well as variations in the composition of milk proteins and fats, may influence the values of mineral substances in the milk (Güler, 2007).

Besides major elements, trace elements were noted in the milk composition, whose concentrations were variable depending on the region from where the milk samples were collected (Table 2). The presence of certain trace elements (Zn, Mn, Fe) in smaller amounts can cause certain health problems since they can be found

in the structure of a large part of the enzyme systems or are present in the internal organs such as liver, kidneys, muscles, bones, or participate in the metabolism of carbohydrates, fats and proteins (Perween, 2015; Ivanova *et al.*, 2011). Also, the presence of certain trace elements (Pb, Cd, Cr) in the organism in higher concentration can be toxic and can have adverse effects on the human health causing serious diseases and damages to the nervous and reproductive systems, and can also contribute to the occurrence of malignancies (Enb *et al.*, 2009; Elbarbary and Hamouda, 2013; Perween, 2015).

In all the examined milk samples the concentration of Ag remained below the limit of detection (< 0.1 mg.kg<sup>-1</sup> wet weight) with the exception of the milk samples taken from the farms in the Sveti Nikole and Bistra regions where the concentration of Ag was 0.12 mg.kg<sup>-1</sup> and 0.32 mg.kg<sup>-1</sup>, respectively. The presence of Ag in the milk samples taken from the farm in Sv. Nikole may be due to the higher concentration of silver in the soil of that region (Stafilov *et al.*, 2014), but the presence of this element in the other milk samples collected from the Bistra region indicates possible contamination of the tools used during milking or milk collection. The processing equipment and milk storage containers can be a source of trace elements in milk also (Yabrir *et al.*, 2014).

The concentration of Al in the milk is also variable, with highest measured concentration observed in the milk samples collected from the Bistra region (2.26 mg.kg<sup>-1</sup>). Compared to the milk samples from

Table 2: Content of trace elements in raw ewes' milk (mg.kg<sup>-1</sup> wet weight)

Region	House hold	Ag	Al	As	Ba	Cd	Co	Cu	Fe	Mn	Ni	Pb	Sr	Zn
Sv. Nikole	1	<0.1	1.43	<0.1	0.162	<0.01	<0.1	1.18	1.59	0.04	<0.1	<0.1	0.72	2.90
	2	0.12	0.93	<0.1	0.251	<0.01	<0.1	1.35	2.02	0.05	0.13	<0.1	0.95	3.99
	3	<0.1	1.01	<0.1	0.220	<0.01	<0.1	1.47	1.98	0.05	<0.1	<0.1	1.10	4.10
	Av ± Sd	nd	1.12 ± 0.27 <sup>b</sup>	nd	0.21 ± 0.04 <sup>ac</sup>	nd	nd	1.33 ± 0.14 <sup>ac</sup>	1.86 ± 0.24 <sup>ac</sup>	0.05 ± 0.01 <sup>b</sup>	nd	nd	0.92 ± 0.19 <sup>acd</sup>	3.67 ± 0.66
Sasa	4	<0.1	1.07	<0.1	0.345	<0.01	<0.1	0.81	1.42	0.05	<0.1	<0.1	0.46	3.35
	5	<0.1	1.89	<0.1	1.559	<0.01	<0.1	1.32	3.82	0.13	<0.1	<0.1	0.87	6.27
	6	<0.1	0.93	<0.1	0.837	<0.01	1.68	0.88	1.70	0.08	<0.1	<0.1	0.82	3.71
	Av ± Sd	nd	1.30 ± 0.52 <sup>b</sup>	nd	0.91 ± 0.61 <sup>b</sup>	nd	nd	1.00 ± 0.27 <sup>bc</sup>	2.31 ± 1.31 <sup>abc</sup>	0.09 ± 0.04 <sup>b</sup>	nd	nd	0.72 ± 0.22 <sup>cd</sup>	4.44 ± 1.59
Veles	7	<0.1	1.90	<0.1	0.229	<0.01	<0.1	1.17	2.63	0.08	<0.1	<0.1	0.65	6.09
	8	<0.1	1.26	<0.1	0.183	<0.01	<0.1	0.98	3.07	0.05	<0.1	<0.1	0.44	3.89
	Av ± Sd	nd	1.58 ± 0.45 <sup>cb</sup>	nd	0.21 ± 0.03 <sup>c</sup>	nd	nd	1.07 ± 0.13 <sup>ac</sup>	2.85 ± 0.31 <sup>abc</sup>	0.06 ± 0.02 <sup>b</sup>	nd	nd	0.54 ± 0.15 <sup>bcd</sup>	4.99 ± 1.56
Manovo	9	<0.1	1.00	<0.1	0.406	<0.01	<0.1	0.83	1.52	0.06	<0.1	<0.1	0.81	4.40
	10	<0.1	1.22	<0.1	0.338	<0.01	<0.1	0.68	2.36	0.07	<0.1	<0.1	0.63	3.12
	Av ± Sd	nd	1.11 ± 0.16 <sup>b</sup>	nd	0.37 ± 0.05 <sup>abc</sup>	nd	nd	0.76 ± 0.11 <sup>bc</sup>	1.94 ± 0.59 <sup>abc</sup>	0.07 ± 0.01 <sup>b</sup>	nd	nd	0.72 ± 0.13 <sup>d</sup>	3.76 ± 0.91
Skopje Bistra	11	<0.1	0.86 <sup>b</sup>	<0.1	0.158 <sup>c</sup>	<0.01	<0.1	0.66 <sup>b</sup>	1.70 <sup>c</sup>	0.05 <sup>b</sup>	<0.1	<0.1	0.37 <sup>bd</sup>	3.22
	12	0.32	2.26 <sup>a</sup>	<0.1	0.382 <sup>abc</sup>	<0.01	0.57	0.93 <sup>bc</sup>	3.38 <sup>b</sup>	0.16 <sup>c</sup>	0.18	<0.1	0.26 <sup>b</sup>	3.89

Av ± Sd - average ± standard deviation, nd - not detected, a, b, c - values in the same column with different letters differ significantly (p < 0.05).

the Sv. Nikole, Sasa, Mariovo and Skopje regions this value is significantly higher at the level of  $p < 0.05$ . The concentration of Al measured in milk samples taken from all the regions showed a significantly greater value compared to the study of Elbarbary and Hamouda (2013), who reported that the raw milk contained  $0.561 \text{ mg.kg}^{-1}$  of Al. The results of this study are in accordance with the results of Güler (2007) and Park *et al.* (2007) according to which the concentration of Al in goat and sheep milk is 3.76 ppm and  $0.05\text{--}0.18 \text{ mg.100 g}^{-1}$ . Most likely the containers and utensils with which the milk comes in contact with are the cause of higher concentration of Al in the milk, also indicated by Coni *et al.* (1996).

The highest concentration of barium was observed in the milk samples collected from the farms in the surroundings of the Sasa mine. These values are significantly higher at level  $p < 0.05$  compared to the values measured in the milk samples collected from the Sveti Nikole, Veles and Skopje regions. The results obtained from this study are similar to the results of Ivanova (2011), but the values are lower compared to the results of Güler (2007) according to which the value of Ba in goat milk is 0.99 ppm. The higher presence of this element in the milk samples obtained from the farms in the vicinity of the Sasa mine may be due to introduction of this element through nutrition or through pasture vegetation. Namely, the mine tailings site is located along the road leading to it, and it is possible that the Ba reaches the vegetation by the wind dust from the tailings. Also, its concentration in the soil is higher compared to the content of Ba in the soil from the other locations (Stafilev *et al.*, 2014).

The results of the Cu, Fe, Mn and Zn concentration measurements are shown in Table 2 with certain variations of these elements in the milk samples collected from different regions. Higher concentration of Cu are observed in the milk samples collected from the farms in the Sveti Nikole, Sasa, Veles and Bistra regions. The content of Cu in the milk samples collected from the farms in the Sveti Nikole region showed significantly higher values ( $p < 0.05$ ) compared to the milk samples taken from the farms in the Sasa, Mariovo, Skopje and Bistra regions. The comparison of Cu concentration in the milk samples taken from the Skopje and Veles regions indicates that the milk sample from the region of Veles contained significantly higher concentration. The results obtained with this study show higher values compared to the results of Borys *et al.* (2006); Park *et al.* (2007); Güler (2007); Yabrir *et al.* (2014) who observed presence of Cu in the amounts of  $0.090\text{--}0.098 \text{ mg.kg}^{-1}$ ,  $0.04 \text{ mg.100 g}^{-1}$ , 0.48 ppm, 0.42–0.47 ppm in ewe and goat milk. The results of this study are similar or the values are slightly higher when compared to the results of Ivanova *et al.* (2011), and lower compared to the results of

Elbarbary and Hamouda (2013). The higher values of Cu in the tested milk are probably due to its naturally higher presence in the soil in the Sv. Nikole region (Stafilev *et al.*, 2014), while the Sasa and Veles regions are affected by the activities of the Pb–Zn Sasa mine (Stafilev *et al.*, 2014; Balabanova *et al.*, 2015) and the smelter (Stafilev *et al.*, 2010), respectively. Besides nutrition, the higher concentration may be influenced by the milk treatment (Enb *et al.*, 2009; Zamberlin *et al.*, 2012).

Iron is a component of the hemoglobin, myoglobin, and other proteins and also a cofactor for many enzymes. Its presence in ewe milk according to Zamberlin *et al.* (2012) is in the range of  $62\text{--}100 \text{ }\mu\text{g.100 g}^{-1}$ , while in buffalo or cow milk it is in the range of  $0.786\text{--}1.242 \text{ mg.kg}^{-1}$  and  $0.607\text{--}0.794 \text{ mg.kg}^{-1}$ , respectively (Enb *et al.*, 2009). The concentration of Fe in the tested ewes' milk is in the range of  $1.42\text{--}3.82 \text{ mg.kg}^{-1}$ , while the highest concentration was observed in the milk samples collected from the farms in the Sasa and Bistra regions. Statistical analysis of data demonstrated significant differences in the content of Fe at the level  $p < 0.05$ , which was observed in the milk samples taken from the farms in the Sveti Nikole and Bistra, and Skopje and Bistra regions. The results of this study show similarities with those of Güler (2007) and Ivanova *et al.* (2011), but have higher values compared to the investigations of De la Fuente *et al.* (1997); Borys *et al.* (2006); Park *et al.* (2007) and Yabrir *et al.* (2014).

The region of Bistra is considered as a region that is not under high anthropogenic influence and there are no urban areas as well as industrial facilities nearby. The high concentration of Fe in the milk samples collected from this region is probably a result of the containers used for milk collection (Güler, 2007). The high concentration of Fe in the milk samples collected from farms in the Sasa region ( $3.82 \text{ mg.kg}^{-1}$ ) and Veles ( $3.07 \text{ mg.kg}^{-1}$ ) region are most likely influenced by the activity of the Sasa mine and the Veles smelting plant. Although the smelter has not been active in the recent years, the soil in its environment has accumulated Fe in higher concentration (Stafilev *et al.*, 2010, 2014; Balabanova *et al.*, 2015).

The content of Mn in milk samples showed the highest value in the milk from Bistra which is significant at level of  $p < 0.05$ , compared to the milk samples from all other surveyed regions. When comparing the results of the tests with those of De la Fuente *et al.* (1997); Park *et al.* (2007); Ivanova *et al.* (2011); Zamberlin *et al.* (2012) and Yabrir *et al.* (2014) some similarities can be observed. Variations in the concentration of mineral components are very characteristic for ewe milk compared to cow milk (Park *et al.*, 2007). The higher concentration of Mn in the milk is affected by the metabolism of metals in sheep (Caggiano *et al.*, 2005), its concentration in the nutrition and the additional intake of minerals from

the metal containers and tools used for milking and storage of milk (Güler, 2007).

The examination of Ni concentration in milk indicates values below the limit of detection ( $<0.1 \text{ mg.kg}^{-1}$ ) in almost all tested milk samples. The samples taken from a farm in the Sveti Nikole region and the farm region of Bistra where the concentration of Ni was  $0.13 \text{ mg.kg}^{-1}$  and  $0.18 \text{ mg.kg}^{-1}$  are exceptions. The occurrence of Ni in these two milk samples probably originates from the containers in which the milk was collected, i.e. kept. According to the study of Lukáčová *et al.* (2012) Ni is present in the raw cow milk at a concentration of  $0.84 \text{ mg.kg}^{-1}$ , while according to Enb *et al.* (2009) cow and buffalo milk contains Ni at concentrations of  $0.004 \text{ mg.kg}^{-1}$  and  $0.006 \text{ mg.kg}^{-1}$ . In goat milk Güler (2007) found a Ni content of 1.38 ppm, and Zamberlin *et al.* (2012) provided data that the Ni content in sheep milk is  $5.4 \mu\text{g.}100 \text{ g}^{-1}$ .

Unlike Ni, strontium was detected in all milk samples taken for analysis. The lowest concentration of Sr was measured in the milk samples collected from the Bistra region which shows significantly lower value ( $p < 0.05$ ) compared to the samples taken from the Sveti Nikole, Sasa and Mariovo regions. The concentration of Sr in the milk from Sveti Nikole was significantly higher than the one observed in the milk samples from Skopje and Veles, as well as the milk samples from the Sasa area in relation to Skopje. The concentration of Sr in milk also depends on its concentration in the pastures' vegetation and the ability of the plants to absorb it. The results obtained from this study indicate lower to similar values compared to the results of Güler (2007) who examined goat milk. The higher content of Sr in milk observed in the course of this study could be generally explained with its lithogenic origin in the soil in the Republic of Macedonia, which has relatively high content of Sr (Stafilov *et al.*, 2014).

The concentration of Zn in the tested samples of milk was in the range of  $2.90\text{--}6.27 \text{ mg.kg}^{-1}$ . From the results, it is evident that the highest concentration of Zn was measured in the milk samples from the farms in the Sasa and Veles regions. The higher level of Zn concentration in the soil is the result of anthropogenic influence (Sasa Pb-Zn mine and Pb-Zn smelter in Veles), since it contributes to increased concentration in the vegetation that serves as fodder for the sheep (Stafilov *et al.*, 2010, 2014; Balabanova *et al.*, 2015). However, statistical analysis of the data showed no significant differences in the concentration of Zn in milk samples from different regions. Elsayed *et al.* (2011) observed higher levels of Zn in the cow milk samples from the industrial regions, but the values are lower than those obtained with this study which is understandable because ewes' milk contains higher concentration of trace elements. The values in this study are lower than

the results of Borys *et al.* (2006); Yabrir *et al.* (2014) but are similar to those of Güler (2007) and Park *et al.* (2007). The values for Zn in cow milk, recommended by the IDF Standard (1977), are lower in relation to the obtained results shown in Table 2. According to the Standard, MRL for Zn in milk is  $0.328 \text{ mg.kg}^{-1}$ .

As for the Co and toxic elements such as As, Cd and Pb, tests showed that in all analyzed milk samples their concentration remained below the limit of detection, namely the concentration of As is  $< 0.1 \text{ mg.kg}^{-1}$ , of Cd  $< 0.01 \text{ mg.kg}^{-1}$ , of Pb  $< 0.1 \text{ mg.kg}^{-1}$ . The concentration of Co was  $< 0.1 \text{ mg.kg}^{-1}$ , with the exception of two farms in the Sasa and Bistra regions where the concentrations were  $1.68 \text{ mg.kg}^{-1}$  and  $0.57 \text{ mg.kg}^{-1}$ , respectively. The concentration of Cr in the milk was not measured because of the added  $\text{K}_2\text{Cr}_2\text{O}_7$ . The higher concentration of Co measured in the milk samples from the two stated farms is probably the result of additional contamination from the dairy equipment because higher concentration of the same elements are not observed neither in the curd nor in the cheese made from the tested milk (Levkov unpublished data). According to Elsayed *et al.* (2011) higher concentration of Pb and Cd in cow milk are observed in the industrial regions. According to the IDF Standard (1977) MRL of Cd in milk from uncontaminated areas is  $0.006 \text{ mg.kg}^{-1}$  while from contaminated areas it is  $0.015 \text{ mg.kg}^{-1}$ . Güler (2007) found a concentration of Pb in goat milk in the amount of  $0.06 \text{ mg.kg}^{-1}$ , Cd  $0.63 \text{ mg.kg}^{-1}$ , Co  $0.89 \text{ mg.kg}^{-1}$  while As was not detected at all. The concentration of Pb of  $0.20 \mu\text{g.g}^{-1}$  dry weight in ewe's milk was observed by Caggiano *et al.* (2005) while the concentration of Cd amounted to  $0.06 \mu\text{g.g}^{-1}$  dry weight. Elbarbary and Hamouda (2013) measured Pb concentration in raw milk in the amount of  $0.27 \text{ mg.kg}^{-1}$  and Cd  $0.053 \text{ mg.kg}^{-1}$ . Higher concentration of Cd ( $0.27 \text{ mg.kg}^{-1}$ ) in raw cow milk was found by Lukáčová *et al.* (2012). According to Caggiano *et al.*, (2005) kidneys and liver are considered bio-accumulators of metals, which are introduced in the bodies of animals through food, so that the same are excreted in the milk in small concentration.

## CONCLUSION

The results of this study showed variations in the concentration of mineral and trace elements in the raw ewes' milk used for production of traditional dairy products. For the purpose of this study, milk samples were collected from households located in different geographic regions characterized by specific soil composition and pasture vegetation. The concentration of trace elements in the soil, the mines and the industrial activities, as well as the utensils and containers used for milk storage influenced the higher concentration

of trace elements (Ag, Al, Ba, Cu, Fe, Mn, Ni, Sr, Zn) in some of the analyzed samples. Milk samples taken from the regions exposed to higher levels of pollution showed that the concentrations of toxic elements were below the limit of detection. The obtained results point out that the ewes' milk produced in households and used for production of traditional dairy products is safe for consumption. In the Republic of Macedonia there is little published data related to this issue of research, therefore it is advisable to conduct more detailed analyses of other types of milk (cow, goat) produced in other geographical regions as well as the fodder that animals consume.

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