HEAVY METAL CONTENTS IN HOMEMADE MILK AND CHEESE FROM VILLAGES WITH POSSIBLE RISK OF ENVIRONMENTAL POLLUTION

Pavlina Gidikova*, Rositza Deliradeva*, Sashka Chobanova**, Gospodinka Prakova*, Gurga Mihailova**

**Faculty of Medicine, Trakia University, 11 Armeiska Str, 6000 Stara Zagora, Bulgaria*
pgidikova@yahoo.com; rdeliradeva@mail.bg; prakova@hotmail.com
*** Faculty of Agriculture, Trakia University, Studentski grad, 6000 Stara Zagora, Bulgaria*
sira@abv.bg; gmih@uni-sz.bg

ABSTRACT

Milk and dairy products are an important food group because of their nutritional features. Unfortunately, milk could be contaminated with heavy metals from the environment. **The aim** of this study was to investigate heavy metals (lead, cadmium and nickel) in homemade milk and cheeses from animals raised in villages with possible risk of pollution - Zmeyovo and Borilovo. **Materials and Methods:** Raw goat and cow milk samples as well as cheese samples were taken from two herds in Zmeyovo, two herds in Borilovo and raw cow milk from remote mountain village was taken as a control. Samples were analyzed using flame and graphite furnace atomic absorption spectrometry after microwave or wet digestion. The results obtained were divided in groups depending on the kind of dairy animals, type of feeding and villages. **Results:** Mean lead contents in milk ranged from 0.022 mg/kg in cow milk to 0.032 mg/kg in milk from grass-fed goats. In all milk samples the lead content was over the maximum permissible level of 0.020 mg/kg determined by Regulation EC 1881/2006. Cadmium and nickel levels were highest also in milk from grass-fed goats and in goats that were fed grass and locally grown barley and alfalfa. Lead and nickel contents in goat cheese were higher than in cow cheese, but cadmium contents were almost equal in all cheese samples. **Conclusions:** Lead levels in homemade milk and cheeses are cause of concern. Risk for heavy metals contamination of dairy products is higher in goat herds with grass pasture feeding.

Key words: heavy metals, milk, cheese

Milk and dairy products are an important food for people of any age because of their nutritious features. They are a good source of easily assimilated proteins, fats, carbohydrates, calcium, phosphorus, potassium, riboflavin, vitamin A, and vitamin D (Bakircioglu et al., 2011; Ibrahim and Mehanna, 2015). Unfortunately, milk could be contaminated with heavy metals from the environment entering the food chain. Inclusion of toxic metals compromises the safety of dairy products. Heavy metals in the food chain come mostly through consumption of plants directly polluted with air or soil particles. Dairy cattle ingest heavy metals also through consumption of grasses or fodder crops contaminated during the vegetation from polluted soil or irrigation waters (De Winter-Sorkina et al., 2003; Järup L, 2003). Persistence in the soil and bioaccumulation in plants are typical for heavy metals (WHO, 2000; WHO, 2007; Jaishankar et al., 2014). Soil and plants can be polluted with heavy metals from various sources such as dry or wet atmospheric deposition, fertiliser application, water and sewage contamination. Dairy products could be contaminated also from the equipment during the processing, packaging, and storage (Bakircioglu et al., 2011).

Environmental pollution with heavy metals is possible in the area of Stara Zagora municipality, due to the presence of potential sources like the biggest in Southeastern Europe coal power complex Maritsa Iztok with surface coal mines and three power stations, the military proving ground Zmeyovo, some metal production and processing plants, a highway and several major roads and transport junctions. In recent years (2005, 2007 and 2008) on behalf of the municipality 141 plant samples were tested for heavy metals contamination by UIS Umweltinstitut Synlab GmbH Stuttgart, Germany. Mushrooms, walnuts, grapes, apples, pears, tomatoes, cabbage were sampled from nine different villages and one location within the town's limits and were tested for seven metals - lead, cadmium, nickel, zinc, arsenic, chromium, copper, manganese, and iron. The highest rate of concentrations exceeding the maximum permissible levels was recorded in two villages situated northwest of Stara Zagora - Borilovo (9.2% excessive and 22.9% borderline concentrations) and Zmeyovo (7.4% and 14.7%, respectively), mainly for lead, cadmium and nickel (Gradeva et al., 2012). No foodstuffs from animals raised in the region were tested for heavy metals content.

The aim of this study was to investigate the contents of heavy metals (lead, cadmium and nickel) in homemade milk and cheese from dairy animals raised in the villages with possible increased risk for contamination – Zmeyovo and Borilovo.

Materials and Methods

Raw goat milk and/or raw cow milk samples were taken from two herds in Zmeyovo, two herds in Borilovo and raw cow milk from remote mountain village was taken as a control. Since the trace element concentrations in raw milk vary mainly according to two kinds of factors, those related with the lactation state, animal species and health status, and extrinsic factors, such as season, nutritional status and environment (Coni et al., 1999; Sola-Larracaga & Navarro-Blasco, 2009), samples were divided in groups depending on the kind of dairy animals, feeding and locality of the farm. Samples were gathered in spring and in summer when the animals were fed predominantly with grass pasture. Barley and alfalfa were given as complementary feeding in two goat herds only. Sampling was made after mixing of milk yield during the morning milking of all animals in the herd. Random cheese samples were collected for each herd in Zmeyovo and Borilovo. Precautions were taken to avoid any changes which would affect the lead, cadmium and nickel contents, adversely affect the analytical determination or make the aggregate samples unrepresentative. All materials used for sampling were nitric acid-washed and rinsed with ultrapure water.

Samples were analyzed using flame and graphite furnace atomic absorption spectrometry (AAS) after microwave or wet digestion. Concentrated HNO_3 (5-10 ml) and concentrated H_2SO_4 (2 ml) were added carefully and the test portion was allowed to stand at room temperature overnight. Then the mixture was heated in a sand bath at a low temperature, ensuring that the sample does not boil. After initial digestion the temperature was increased and the heating continued until the solution became colorless. Concentrated nitric acid was added to avoid the reduction of volume. Because of the high fat content, 1 mL of concentrated $HClO_4$ was added to the solution. After decoloration the solution heating continued until the volume was reduced to 2-3 ml. The remainder of about 2-3 ml was filtered and quantitatively transferred to a 10 ml graduated test tube with 0.2 N nitric acid. Microwave digestion was performed in microwave oven MULTIWAVE 3000 with nitric acid, hydrogen peroxide and hydrochloric acid. All the chemicals used were of the highest purity available. Atomic absorptions for lead, cadmium and nickel were measured by ANALYST 800 AA SPEKTROMETER, Perkin Elmer, working with flame or graphite tubes. The concentrations of Pb, Cd and Ni were calculated using calibration curves, drawn through measuring of the corresponding standard solutions with appropriate dilution. Precision of the measurement was tested by ten-fold investigation of one and the same sample. The variance for Pb, Cd and Ni were $0.05x10^{-6}$, $0.03x10^{-6}$ and $0,04x10^{-6}$ respectively, that were expected to result in very narrow deviation of the measurements. Standard error was estimated as $0.073x10^{-3}$ for lead, $0.053x10^{-3}$ for cadmium and $0,065x10^{-3}$ for nickel.

The results obtained for groups based on the kind of dairy animals, feeding and villages were compared with each other and with the corresponding maximum permissible levels, determined by Commission Regulation (EC) No 1881/2006. Although under the Regulation (EC) No 853/2004 community rules should not apply either to primary production for private domestic use or to the domestic preparation, handling or storage of food for private domestic consumption, the regulated maximum permissible levels could be used for comparison.

Results and discussion

Mean concentrations of lead, cadmium and nickel estimated in milk and cheese samples with different origin are presented in Table. 1.

Table 1. Heavy metalslevels in milk and homemade cheese from dairy livestock raised in Stara Zagora region.

Product – Village – Feeding of the dairy animals	Concentrations of heavy metals (mg/kg wet weight)		
	Pb	**Cd**	**Ni**
Cow milk – Zmeyovo – Grass pasture	0.022	0.0082	0.097
Goat milk – Zmeyovo – Grass pasture	0.032	0.0093	0.098
Goat milk – Borilovo – Grass pasture and complementary feeding with locally grown barley and alfalfa	0.027	0.0090	0.108
Goat milk – Borilovo – Grass pasture and complementary feeding with barley and alfalfa from remote region	0.024	0.0076	0.094
Cow milk – Kolio Marinovo – Grass pasture	0.024	0.0077	0.105
Cow cheese – Zmeyovo – Grass pasture	0.477	0.052	0.205
Goat cheese – Zmeyovo – Grass pasture	0.489	0.051	0.223
Goat cheese – Borilovo – Grass pasture and complementary feeding with locally grown barley and alfalfa	0.493	0.049	0.225
Goat cheese – Borilovo – Grass pasture and complementary feeding with barley and alfalfa from remote region	0.496	0.050	0.219

Lead levels (Table 1) measured in goat milk samples were higher than those in cow milk. The highest Pb concentration was measured in milk from grass-fed goats from the region of Zmeyovo. Second highest was for the goats from Borilovo fed grass and locally grown barley and alfalfa. In milk from goats complementary fed with barley and alfalfa from remote region lead content was equal to the control. This suggests that grass and cultivated plants from the region contain lead in increased levels. In all milk samples the lead content was over the maximum permissible level of 0.020 mg/kg determined by Regulation EC 1881/2006, indicating an increased background lead pollution in the environment of grazing livestock.

Lead in goat cheese was significantly higher ($p<0.005$) than in cow cheese, just as with milk. The Pb levels in cheese from different goat herds did not vary considerably. It was interesting to observe that Pb content in cheese was about twenty-fold higher than in milk from the same heard. An explanation could be that homemade cheese was produced only from milk without any supplementation or additives. Vural et al. (2007) have estimated a significant positive correlation between lead concentration and rate of dry matter in herby cheese from Anatolia-Turkey. Tahvonen and Kumpulainen (1995) in their study also found ten or more times higher lead concentrations in Finish cheese than in milk.

Cadmium levels (Table 1) were highest also in milk from grass pastured goats in the area of Zmeyovo, followed by the herd from Borilovo fed with grass and fodder crops grown in the region. Mean Cd level in cow milk from Zmeyovo was also higher than in the control cow milk. A combined contamination of the area with lead and cadmium was suggested. This was confirmed by the significant positive correlation between blood lead levels and blood cadmium levels found in our previous investigation among villagers from Zmeyovo and Borilovo (Gidikova et al., 2015). Cadmium levels in homemade cheeses from the studied two villages were similar.

As could be seen on the Table 1, nickel levels in milk showed different results. The highest level of Ni was measured in milk from Borilovo herd fed with locally grown grass, barley and alfalfa. Moreover, this level exceeded recommended maximum permissible of 0.1 mg/kg. As with the lead, nickel content in goat cheese was significantly higher than in cow cheese ($p<0.05$).

In literature about heavy metals contamination of milk and cheese different levels for different metals were reported. A wide variety of concentrations was reported in a study on levels of metals in cow's milk and dairy products from Palestine market. In milk samples from 21 brands (14 local and 7 foreign) lead levels ranged from 0.07 mg/kg to 0.61 mg/kg, but cadmium levels ranged from 0.001 to 0.172 mg/kg. In white cheese lead was not detected and cadmium levels varied from 0.002 mg/kg to 0.130 mg/kg (Abdulkhaliq, 2012). Licata et al. (2004) investigated 40 samples of cow milk from various dairy farms in Calabria, Italy. They found much lower concentrations but significant variations. Lead levels were in the range 0.0001-0.0099 mg/kg w.w. raw cow milk. In 37 samples cadmium concentration was under the detection limit of 0.01 μg/kg and in the other three samples ranged from 0.0011 mg/kg to 0.0228 mg/kg.

Karadjova et al. in 2000 measured heavy metal concentrations in store bought white cheese using electrothermal atomic absorption spectrometry after wet digestion. They found Pb concentrations (0,028 mg/kg) about seventeen fold lower than our results for homemade cheese, Cd concentrations (0.027 mg/kg) two fold lower and Ni concentration (0.260 mg/kg) similar to our result. Gogoasă et al. (2006) measured metal content in sheep cheese samples from three mountain and hill areas in the Banat, Romania and found similar to our concentrations for lead (0.193-0.314 mg/kg) and much lower concentrations for cadmium (0.001-0.003 mg/kg) and nickel (0.002-0.010 mg/kg). As already mentioned, heavy metal contents depend on animal species, feeding, season, environmental conditions and manufacturing processes (Yuzbasi et al. 2003; Caggiano et al. 2005). Yüzbaşi et al. (2003) measured lead, cadmium, iron, copper and zinc contents of Kaşar cheese sold in the markets of Ankara, Turkey over 12 months and found Pb and Cd concentrations in very wide range – from 0,010 to 0,421 mg/kg for lead and from 0.0003 to 0.0083 for cadmium. The highest results for lead in cheese were similar to ours, but cadmium contents were many times lower. On the other hand, Vural et al. (2007) found lead contents in herby cheese in very big range of 1.5–10.7 mg/kg, which were much higher than measured in our samples. Cadmium (0.1-0.6 mg/kg) and nickel (0.8–4.8 mg/kg) levels were found also in wide range and all concentrations were higher than measured by us. Herby cheese is manufactured at home in villages and in some small workshops in south eastern Anatolia-Turkey. With semi-hard texture and salty taste this homemade cheese is similar to our samples. In Turkish white cheese Orak et al. (2005) found cadmium (0.127 mg/kg) and nickel (1.057 mg/kg) concentrations also higher than ours. In another study on Turkish herby cheese Mendil (2006) estimated mean lead level as 0.32 mg/kg. Very similar to our results for lead levels in cheese were reported from Orak et al. (2005) - 0.415 mg/kg in Turkish white cheese and from Caggiano et al. (2005) - 0.35–0.58 mg/kg range in cheese samples from Southern Italy. Nickel in herby cheese from Van-Turkey estimated by Mendil (2006) was almost equal to ours- 0.22 mg/kg.

Conclusions: Lead levels in milk and homemade cheeses from dairy livestock grown in the Stara Zagora region are a cause of concern. Risk of heavy metal contamination is higher in goat homemade dairy products from herds fed with grass pasture or complementary fed with fodder crops grown in the region with possible environmental pollution.

Acknowledgements: This study was supported by a grant (13/2015) from the Faculty of Medicine, Trakia University, Bulgaria. The authors wish to thank all farmers who submitted milk and cheese samples.

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