Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2017, VIII(16)

Physical–chemical parameters and the level of heavy metals in cow milk in the Baia Mare area

DOI: 10.7904/2068–4738–VIII(16)–69

Aurelia COROIAN[1], Vioara MIRESAN[1], Daniel COCAN[1], Camelia RADUCU[1], Adina Lia LONGODOR[1], Alexandra POP[1], Gratian FEHER[1], Luisa ANDRONIE[1], Zamfir MARCHIS[1*]

*[1]University of Agricultural Sciences and Veterinary Medicine Cluj–Napoca, Faculty of Animal Science and Biotehnology, Manastur Str. 3–5, 400272, **Cluj–Napoca**, **ROMANIA***
Corresponding author: zamfir.marchis@usamvcluj.ro

Abstract. Heavy metals are important pollutants in the environment and can cause problems for the organisms, and the bioaccumulation of heavy metals in the food chain can have adverse effects on human health. Regulations on the legally accepted amounts of heavy metals in food and in the body are very precise. The purpose of this paper was to evaluate the level of heavy metals in cow milk from different localities in the Baia Mare area, considered a very polluted area. The physical–chemical parameters and heavy metals in milk were determined. The determination of heavy metals in milk was performed by inductively coupled plasma mass spectrometry or ICP–MS used to identify and quantify the Pb, Cd, Cu, and Zn elements. The highest concentrations of Pb were observed in samples collected in Firiza locality with an average value of (43.22±0.62) (µg/L) and the lowest concentrations are for the samples analyzed in Săcălăşeni (11.53±0.33) (mg/L) (µg/L). Cd in milk did not show very large variations between localities, so the lowest concentrations were recorded in Săcălăşeni (4.32±0.13) and the highest ones were observed in the locality Firiza (10.93±0.39) (µg/L). The highest concentrations were observed for the Zn in milk, with an interval between (2255.20±7.14) (µg/L) in Săcălăşeni and (3855.80±5.11) (µg/L) for the analyzed samples from Firiza.
Keyword: milk, fat, proteins, Zn, Cd, Cu, Pb.

Introduction

Heavy metals are potential contaminants for the environment and for human health, and can cause problems for the consumer. At this moment, there is an increased attention to metals across the world, as they can have toxic effects even at relatively low levels [DAS, 1990].

Thus, there have been reports of various human diseases, disorders, malformations due to their toxicity [JARUP, 2003]. Mercury, cadmium, copper are heavy metals that pollute the environment, and their presence in water, soil and atmosphere can negatively impact all organisms through bioaccumulation in the food chain. These compounds can become extremely dangerous for the human body. Ingestion is the primary route of exposure to these elements within the population [EJAZUL *et al.*, 2007].

Cd is an element very often associated with Zn and Cu but is extremely harmful to living organisms [BERNHOFT, 2013]. Commercially we can purchase various Cd–containing objects such as: TV screens, batteries, lasers, cosmetics, various paint categories [ROLLA *et al.*, 2012]. Pb is a very widespread, toxic metal, which is why its concentrations in the nature must be constantly monitored.

Sources of Pb contamination are: ducts and pipes used in household or sanitary installations, batteries, various cables used in installations.

There are also additives in fuels, with serious consequences on water pollution [HERNBERG, 2000].

For some heavy metals, the cumulative effect is the most significant property, and this effect is met at Hg, Cd, Pb. Their toxicity occurs at the time of accumulation of a certain amount that causes extremely serious illnesses and irreversible consequences [HELFERICH and WINTER, 2001].

Previous studies have highlighted that the accumulation of all toxic elements in the mining processes taking place in the Baia Mare area, especially Pb which accumulated in the human, animal and vegetal organisms, causing acute, chronic, and cumulative intoxications manifested by the growth of mortality, decrease in the birth rates, increase in the incidence of reproductive disorders, toxic actions at the subcellular level, alteration of the functions and structures of the cellular organelles of the enzymes underlying metabolism and organic biosynthesis, but also through chromosomal destructions or alterations culminating in the occurrence of term mutagenic and teratogenic effects [IVAN *et al.,* 2013]. Unlike As, Cd does not produce methylation, oxidation or reduction, but has a great affinity for anionic groups; Thus, Cd is linked to various proteins in the body such as albumins [PAN *et al.,* 2010].

As a result of Cd contamination, it is excreted through the faeces, and it is not absorbed in the digestive tract. It has high affinity for the liver, kidneys, muscle tissue, skin and bones. Out of the human body, it is eliminated in about 26 years, from the kidneys, the elimination time range varies from 6 to 38 years, and from the liver it is eliminated in a time ranging of 4 to 19 years [SHAIKH, 1980].

Some studies report the occurrence of different cancers due to Cd poisoning such as prostate cancer, breast cancer, lung cancer, bladder cancer [KELLEN, *et al.,* 2007]. Exposure can be through food or contaminated water, but it can also be by contact with products containing Pb, soil or dust particles. Since many plumbing installations are made with Pb pipes, the risk of water contamination is extremely high, but exposure can also be by inhalation of amounts of Pb. These metals can form various complexes with proteins in the body or other compounds that are metabolised in the liver. The elimination of Pb from the body can be achieved through urine and faeces, and in very small amounts they are found in hair, nails, saliva, human milk or sweating [DALE, 1994, HSU, 1981].

It is important to note that industrial activities are the most dangerous causes of heavy metal pollution, as large quantities of heavy metals accumulate in mining, after energy and fuel production, industrial water and nuclear industries [REIMANN and CARITAT, 2005].

The long–term result is that they are found in the air, soil and water, and hence the vast majority of health problems. Heavy metals are one of the most serious causes of illness [DASH, and DAS, 2012, DASH *et al.,* 2013].

Once these elements arrive in the main feed sources, it is very easy for them to reach all food chains, thus affecting the entire food chain.

In addition to this we can recall the risk and the effects of the bioaccumulation of heavy metals in the trophic chain organisms, and the studies show that the human body, the last link of the trophic chain, holds the highest quantities of heavy metals due to the bioaccumulation effect [VINODHINI, and NARAYANAN 2008].

Cu is found in certain foods in larger quantities: meat, eggs, nuts, seeds and cereals, and in low amounts in fruits.

Refined food is poor in Cu and many other minerals. Children need more Cu than adults because of their more active metabolism.

Cu is a metal that occurs naturally in the environment in rocks, soil, water and air. Cu is an essential element for plants and animals (including humans), which means it is a vital element.

Therefore, plants and animals must absorb the Cu from food, drink, and breathing. Cu compounds can also be used as pesticides (Cu pentahydrate sulphate–bloom stone, Cu acetoarsenite–Paris green, *etc*.), Egyptian blue, Voroneţ blue, pigments used in lithography and pyrotechnics. Their uses also extend to the food industry as colouring agents [GILBERT, *et al.,* 2003; TUMOLO and MARQUEZ 2012].

The purpose of this paper was to evaluate the level of heavy metals and physico–chemical parameters in milk from different localities in the Baia Mare area.

Material and methods

Milk samples were collected in sterile containers and stored in the refrigerator until the analysis was performed. The following parameters

Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2017, VIII(16)

were analyzed: fat, protein, lactose, dry matter. A total of 10 samples of milk have been taken from each locality.

The physico–chemical analysis of the milk was performed with the Lactoscan device.

Mineralization of the milk samples was performed as follows: 1 mL sample (milk) was subjected to microwave digestion with 8 mL 65 % HNO_3 and 2 mL 30 % H_2O_2. After cooling to ambient temperature, the milk sample was diluted with 25 mL of ultra–pure water, and then filtered through a 0.45 µm cellulose membrane filter. A Berghoff MWS–3 Microwave Digestor (Eningen, Germany) was used as instrumentation. The determination of heavy metals in milk was performed by inductively coupled plasma mass spectrometry or ICP–MS (Perkin–Elmer ELAN DRC II ICP–MS spectrometer), used to identify and quantify Pb, Cd, Cu, and Zn elements.

Results and discussion

Table 1 shows the average values and variability for fat, protein, lactose and dry matter (DM) in cow milk from the Baia Mare area.

The average content of dry matter varied as follows: the lowest content of DM % was for the milk in Lăpuşel, with an average value (12.61 ± 0.02 %) and the highest for the milk in Baia Mare with a mean value of (12.98 ± 0.02 %).

Table 1.

Physico–chemical parameters of cow milk

Locality	D.M. % X±sx	V%	Fat % X±sx	V%	Proteins % X±sx	V%	Lactose % X±sx	V%
Buşag	12.64±0.03	0.62	3.62 ±0.04	2.46	3.30±0.03	2.14	4.74±0.02	1.16
Lăpuşel	12.61±0.02	0.28	3.84±0.02	1.43	3.28±0.02	1.36	4.62±0.02	0.97
Săcălăşeni	12.68±0.01	0.20	3.88±0.02	1.15	3.26±0.04	2.74	4.54±0.05	2.51
Firiza	12.87±0.03	0.56	3.98±0.02	1.12	3.34±0.04	2.68	4.36±0.04	2.05
Baia Mare	12.98±0.02	0.29	4.12±0.02	1.09	3.38±0.02	1.32	4.48±0.04	1.87

(All values are mean±sx, n = 10)

The fat in the milk showed a range of the following values: (3.62 ± 0.04 %), for the milk in Buşag locality and (4.12 ± 0.02 %) for the milk from Baia Mare. The results obtained for this parameter are within the standard milk values. These values are consistent with those reported by other authors [VELEA and MARGINEAN, 2012, RADUCU *et al.*, 2009] for the fat in cow's milk.

The milk protein is in the range (3.26 ± 0.04 %) for the milk in Săcălăşeni and (3.38 ± 0.02 %) for milk in Baia Mare.

Protein content ranges from the values reported in the cow milk literature [MIRESAN *et al.*, 2009; VELEA and MARGINEANU, 2012].

Protein varies depending on many factors including diet, season, stabulation, grazing, lactation, age. Lactose varied in the range (4.36 ± 0.04 %) for the milk in Firiza and (4.74 ± 0.02 %) for the milk in Buşag. These data are consistent with those reported by [VELEA and MARGINEANU, 2012].

Based on the physico–chemical analysis of milk, it is found that it has average values that fit milk to the standards required by current legislation for fat, protein, lactose and dry matter.

Active milk components as well as those shown in Table 1 can be influenced by the content of contaminants in milk, especially heavy metals which, by their nature, enter the body through milk consumption and cause irreversible changes [FARREL, *et al.*, 2010].

Table 2.

Concentrations of Pb, Cu, Cd, Zn in raw milk samples (µg/L wet weight)

Analized paramater	Buşag locality $X±s_x$	V%	Lăpuşel locality $X±s_x$	V%
Pb	36.68±5.94	6.10	23.94±1.39	12.97
Cu	344.20±3.12	2.03	95.36±1.69	3.95
Cd	5.30±0.21	8.71	5.15±0.10	4.55
Zn	3227.20±3.76	0.26	2867±15.85	1.24

(All values are mean ± sx, n = 10)

Fat besides its energy role also contributes to the formation of fat reserves in the body. Vitamins contained in appreciable proportions raise the nutritional value of milk [BANU, 2007].

Table 2 shows the average values and variability for Pb, Cd, Zn and Cu in milk, in the locality of Buşag and Lăpuşel.

The highest concentrations of Pb were observed in the samples collected from the locality of Firiza, with an average value of (43.22±0.62) (µg/L) and the lowest concentrations are for samples analyzed in Săcălăşeni (11.53±0.33) (mg/L) (Table 3).

Table 3.

Concentrations of Pb, Cu, Cd, Zn in raw milk samples (µg/L wet weight)

Analized pamateter	Săcălăşeni locality		Firiza locality		Baia Mare	
	$X \pm s_x$	V%	$X \pm s_x$	V%	$X \pm s_x$	V%
Pb	11.53±0.33	6.31	43.22±0.62	3.21	30.04± 0.92	6.86
Cu	175.2±3.26	4.16	419.60±4.27	2.28	277.40±3.25	2.62
Cd	4.32±0.13	6.73	10.93±0.39	7.92	6.69±0.20	6.68
Zn	2255.20±7.14	0.71	3855.80±5.11	0.30	2577.80±6.22	0.54

(All values are mean ± sx, n = 10)

The highest concentrations were observed for the zinc, with an interval between (2255.20 ± 7.14) (µg/L) in Săcălăşeni and (3855.80 ± 5.11) (µg/L) for the analyzed samples from Firiza.

The existent data until the present that refers to the zinc effect over the organism shows that it does act on humans and animals over the same target organs [BLANC *et al.*, 1991; BROWN, 1988; HOFFMAN *et al.*,1988]. Table 3 presents the average values and variability for Pb, Cu, Cd and Zn in the analyzed cow milk from Săcălăşeni, Firiza and Baia Mare.

The administration of long–term (1–8 years) treatments with zinc supplements caused anaemia in humans [SALZMAN *et al.*, 2002; RAMADURAI *et al.*,1993]. Even short–term administration over a 10–month period and 2 mg/kg/day (as zinc sulphate) led to anaemia [HOFFMAN *et al.*, 1988].

It has been reported that ingested metals (Pb, Hg, Cd), more than half, come from foods of vegetable origin (vegetables, fruits, cereals).

There are several factors that influence the toxicity threshold of heavy metals in the soil–culture system, such as soil type, soil pH, organic matter content, and other chemical and biochemical soil parameters.

At present, it is intended to apply agronomic practices in order to minimize the availability of heavy metals in the soil.

These practices aim to use organic matter and change the pH. Such programs can be used in certain areas where heavy metals pollution is not expanded [EJAZUL *et al.*, 2007].

Cu can be found in plants and animals and at high concentrations in mussels and oysters. Cu is also found in certain concentrations in many foods and beverages we consume, including drinking water. Certain foods have high Cu content (crustaceans, organs, drinking water). Cu is also used to produce pesticides and fungicides [WHO, 1997].

Another study analyzing Cd, Cu, Pb and zinc on cow milk from Egipt revealed, that milk was found to contain iron (16.38 µg/g), zinc (10.75 µg/g), Pb (4.404 µg/g) and the lowest amount of Cu (2.836 µg/g) and Cd (0.288 µg/g).

The conclusion of this study was that the heavy metals taken in the analysis were present in milk at concentrations much higher than the accepted limits by the standards in force [MALHAT, *et al.*, 2012].

Following analyzes, Pb concentrations were at the upper limit accepted by legislation (0.02 mg/kg), and in the human body the amount of Pb ingested corresponds to 40 % of the permissible daily amount.

Naturally, milk and dairy products have low amounts of iron, zinc or Cu, but in the case of long–term consumption of contaminated milk, the bioaccumulation effect is felt [ARAFA *et al.*, 2014].

Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2017, VIII(16)

The level of heavy metals in milk is greatly influenced by the outdoor environment, the polluted area and the feed administrated to the cows during ~~in~~ lactation.

As a result of the analysis of heavy metals in milk, it can be noticed that in the Baia Mare area and nearby localities the level of heavy metals is high, so they are found in raw milk, due primarily to the intense pollution in this area.

Conclusions

The highest concentrations of Pb were observed in the samples collected from the locality of Firiza, with an average value of 43.22 ± 0.62 (μg/L) and the lowest concentrations are for the samples analyzed in Săcălăşeni (11.53 ± 0.33) (mg/L).

Regarding the Cu concentrations, very large variations can be observed between the localities, as follows: Lăpuşel milk samples showed the lowest average values (95.36±1.69) (μg/L) compared to Firiza where there were signalled the highest concentrations (419.60 ± 0.27) (μg/L).

The high concentrations of Pb, Cu, Cd, Zn in raw milk are probably due to the soil and water in the sample area, which is considered to be a very polluted area with toxic metals.

References

1. Arafa, M.S.; Walaa, M.; Moselhy, A.; Hassan, N.Y. Heavy metals and trace elements levels in milk and milk products, *Food Measure*, **2014**, (8), 381–388.
2. Banu, C. Suveranitate, securitate si siguranţa alimentara, *Editura ASAB, Bucuresti*, **2007**.
3. Bernhoft, R.A. Cadmium toxicity and treatment. *The Scientific World Journal*, **2013**.
4. Blanc, P.; Wong, H.; Bernstein, M.S. An experimental human model of metal fume fever. *Annals of Binternal medicine*, **1991**, 114, 930–936.
5. Brown, J.J. Zinc fume fever, *British Institute of Radiology*, **1988**, 61, 327–329.
6. Dale, L.S. Percutaneous absorption of inorganic lead compounds, *Science of the total environment*, **1994**, 145, 55–70.
7. Das, A. Metal ion induced toxicity and detoxification by chelation therapy. In: 1st(ed) *A text book on medical aspects of bioinorganic chemistry, CBS, Delhi*, **1990**, 17–58.
8. Dash, H.R.; Das, S. Bioremediation of mercury and importance of bacterial *mer* genes. *International biodeterioration & biodegradation*, **2012**, 75, 207–213.
9. Dash, H.R.; Mangwani, N.; Chakraborty, J.; Kumari, S.; Das, S. Marine bacteria: potential candidates for enhanced bioremediation. *Applied microbiology and biotechnology*, **2013**, 97, 561–571.
10. Ejazul, I.; Xiao–e, Y.; Zhen–li, H.; Qaisar, M. Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. *Journal of Zhejiang University. Science. B*, **2007**, 8(1), 1–13.
11. Farrell, M.; Perkins, W.; Hobbs, P.; Griffith, G.; Jones, D. Migration of heavy metals in soil as influenced by compost amendments. *Environmental pollution*, **2010**, 158, 55–64.
12. Salzman, J.; Hunter, Z.D. International Environmental law and policy (*Edition Fundation Press, New York*), **2002**.
13. Malhat, F.; Hagag, M.; Saber, A.; Fayz, A.E. Contamination of cows milk by heavy metal in Egypt, **2012**.
14. Pan, P.J.; Voulvoulis J.A.; Oates N.; Ihlenfeld, C. Cadmium levels in Europe: implications for human health. *Environmental geochemistry and health*, **2010**, 32, 1–12.
15. Ramadurai, J.; Shapiro, C.; Kozloff, M.; Telfer, M. Zinc abuse and sideroblastic anemia, *American journal of hematology*, **1993**, 42, 227–228.
16. Raducu, C.; Miresan, V.; Pece, A.; Coroian, C.; Pusta, D.; Negrea, O. Results regarding the effect of various factors on the main quantitative and qualitative traits of milk to a population of Romanian cow breed. *Lucrari stiintifice Zootehnie si Biotehnologii*, **2009**, 42 (2).
17. Reimann, C.; Caritat, P. Distinguishing between natural and anthropogenic sources for elements in the enviroment: regional geochemical surveys versus enrichment factors,

Science of the Total Environment, **2005**, 337:91–107.
18. Gilbert, B.; Denoël, S.; Weber, G.; Allart D. Analysis of green copper pigments in illuminated manuscripts by micro Raman spectroscopy". *Analyst*, **2003**, 128, 1213–1217.
19. Helferich, W.; Winter, C.K. Food Toxicology, CRC Press LLC, **2001**.
20. Hernberg, S. Lead poisoning in a historical perspective, *American Journal* of *Industrial Medicine*, **2000**, 38, 244–254.
21. Hoffman, H.N.; Phyliky, R.L.; Fleming, C.R. Zinc–induced copper deficiency. *Gastroenterology*, **1988**, 94:508–512.
22. Hsu, J.M.; Lead toxicity as related to glutathione metabolism, *Journal of Nutrition*, **1981**, 111, 26–33.
23. Ivan, I.; Muresan, L.; Deac, T. The study of heavy metals level from ambient air: case study–Cluj County, Proceeding of the 41. *International Symposium on Agricultural Engineering*, Opatia, Croatia, **2013**, 1333–2651.
24. Järup, L. Hazards of heavy metal contamination, *British Medical,* **2003***,* 68:(1), 167–182.
25. Järup, L. Hazards of heavy metal contamination. *British Medical Bulletin*, **2014**, 68, 167–182.
26. Kellen, E.; Zeegers, M.P.; Hond, E.D.; Buntinx, F. Blood cadmium may be associated with bladder carcinogenesis: the Belgian case–control study on bladder cancer, *Cancer detection and prevention*, **2007**, 31 (1), 77–82.
27. Miresan, V.; Raducu C.; Daraban, S.; Pece, A.; Coroian, C. The evidence of the main characteristics of cow milk in function of quality class. *Lucrari stiintifice Zootehnie si Biotehnologii*, **2009**, 42 (2).
28. Rolla, M. U.S. Geological Survey, Mineral Commodity Summaries, *U.S. Geological Survey*, USA, **2012**.
29. Shaikh, Z.A.; Smith, J.C.; Metabolism of orally ingested cadmiun in humans, *Developments in Toxicology and Environmental Science*, **1980**, 8, 569–574.
30. Tumolo, T.; Marquez, U.M. Copper Chlorophyllin: a food colorant with bioactive properties. *Food Research International*, **2012**, 46(2), 451–459.
31. Velea, C.; Marginean G. Tratat de cresterea bovinelor, *Editura Risoprint, Cluj–Napoca*, **2012**, 484–486.
32. Vinodhini, R.; Narayanan, M. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (*Common carp*)*, International Journal of Environmental Science* and *Technology*, **2008**, 5, 179–182.
33. WHO, Environmental Health Criteria, Methanol. *World Health Organization, Geneva*, **1997**.

Received: May 23, 2017
Article in Press: October 27, 2017
Accepted: Last modified on: November 20, 2017