HUNGARIAN JOURNAL
OF INDUSTRIAL CHEMISTRY
VESZPRÉM
Vol. 39(1) pp. 15-19 (2011)

THE EFFECT OF HIGHWAY TRAFFIC ON HEAVY METAL CONTENT OF COW MILK AND CHEESE

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Human exposure to heavy metal elements through milk and dairy products may have influences on food safety. Although certain elements such as zinc, chromium are essential in small quantities, in high amount they can result in toxic effects on animal and human health. In this experiment samples of raw cow milk were collected from dairy farms close to highways in the central region of Hungary, and in rural green regions in West Hungary. Concentrations of selected heavy metals such as As, Cd, Cr, Cu, Fe, Mn, V, Ni, Pb and Zn in cow milk and cheese were determined using inductively coupled plasma mass spectrometry and inductively coupled plasma-optical emission (spectrometry). Chrome, copper, iron, vanadium, manganese, cadmium, arsenic, and lead content showed higher concentrations in the milk from intensive traffic areas than those in the milk from non-polluted green areas. However, in the cheese only copper, chrome, iron and lead concentrations were found significantly higher in the highway samples than those in non-polluted green samples.

Keywords: food safety, dairy products, heavy metal pollution

Introduction

The rapid development of industrial production and traffic system may result in the contamination of food sources. Pesticides, industrial by-products, fertilizers and the increasing number of vehicles using highways, roads are important sources of heavy metals. The potential environmental factors affecting animal production (soil, water and air) are polluted with toxic metals [8] as well. Different levels of these elements have been measured in many countries in soil, vegetation, and the atmosphere. Lead, cadmium, nickel, and zinc concentrations in earthworms were found high by Scanlon [16], mushrooms tended to accumulate some metals [12]. Transport of heavy metal contaminants in the air has been also observed [17]. According to Coni et al. [3] different environmental conditions, rather than animal species, tend to affect the concentration of certain elements in three types of milk (sheep, goat and cow).

Heavy metals are well known, mostly because of potential hazards to the health of living organisms. They can accumulate in tissues of the body, can be toxic even at low level of exposure [11] and can be transferred through food chains. Interest in this subject has led some scientists to the use of plants to clean up contaminated soil and water [15, 19] or to the use of zeolites to remove metals from wastewater [10].

Milk and milk products are important as human foodstuffs; they are essential sources of protein, fat and minerals. The principal type of milk used throughout the world is cow milk. Dairy cattle are fed mostly locally grown forages and preserved feedstuffs and therefore they are exposed to metal contamination if the field is located nearby an industrial area or highway.

This is the reason why the concentrations of certain metals in dairy cattle products have been measured from in several countries [18, 4, 6, 5].

The objective of this study was to study the toxic metal concentrations in cow milk and cheese from two different environmental regions in Hungary. The studied regions included polluted industrial and traffic intensive areas as well as in green non-polluted areas.

Materials and methods

Sample collection and preparation

Cow milk was collected at different dairy farms during the summer of 2011. Two farms were located in polluted areas (near highways M6 and M7; central Hungarian region), where density of the road traffic was high. The other two sampling areas were located in non-polluted green areas (Őrség and Hetés, West Hungary), where the number of roads and traffic density were very low. Mixed milk samples starting from morning milking were taken from each cow.

Cows were fed twice daily with a total mix ratio (TMR) diet formulated to meet the requirements of lactating cows [14]. TMR consisted of corn silage, grass hay, and cereals produced in the indicated areas respectively as main components. Heavy metal contents of the ingredients were measured by the methods indicated later. The concentrations of the metals in the feed ingredients are shown in *Table 1.*

Table 1: Heavy metal contents (mg kg^{-1}) in the ingredients of the diet fed with lactating cows kept in non-polluted green or highway conditions

Non-polluted green area			
Elements	Corn silage	Grass hay	Cereals
Zn	9.33	6.31	13.11
V	0.09	0.07	ND
Cd	ND	ND	0.02
Cr	0.10	0.09	0.10
Pb	0.12	0.10	0.01
Ni	0.42	0.62	0.21
Highway area			
Elements	Corn silage	Grass hay	Cereals
Zn	14.61	6.82	13.81
V	0.15	0.09	0.09
Cd	0.02	0.02	0.02
Cr	0.23	0.23	0.01
Pb	0.22	0.20	0.14
Ni	0.42	0.81	0.22

ND: not detectable

Milk of ten cows was collected at each farm (a total of 40 samples) into plastic tubes. Aliquot volumes of milk were carried separately from each farm to a milk processing factory (Pannontej Zrt.) where a popular Hungarian cheese type (*Trappista*) was produced from the cow milk according to the technology of the factory. After the second month of cheese processing, 400 g samples were collected from each cheese roll respectively and the samples were packed in polyethylene bags. Both milk and cheese samples were stored refrigerated until chemical analyses.

Analytical measurements

All sample treatment and analytical processes were performed in clean conditions to avoid exogenous contamination. Milk, cheese and dietary ingredients were digested by acidic digestion in microwave oven (Ethos 1, *Milestone*).

Approximately 0.5 g of both cheese and feed, and 5 mL of milk samples were transferred into teflon vessels and 8 mL of nitric acid (HNO_3, 65% Suprapur®, Merck) and 2 mL of hydrogen peroxide (H_2O_2, *Trace*SELECT® Ultra) were mixed. Details of the microwave degradation conditions are shown in *Table 2*.

Table 2: Microwave digestion program of the milk, cheese and feed samples

	Program step	Time (min)	Power (W)	Temp. (°C)
Milk, cheese	1	03:00	800	85
	2	05:00	800	145
	3	05:00	800	180
	4	12:00	800	200
	Program step	Time (min)	Power (W)	Temp. (°C)
Feed	1	03:00	800	85
	2	04:00	800	145
	3	07:00	800	200
	4	11:00	800	200

Microwave degradation was followed by a cooling period at room temperature, and then the sample solutions were transferred into volumetric flasks and adjusted to 25 mL up with high purity, double deionised water. The digested solutions were then stored at room temperature until analyses. Blank digest was made in the same way without milk, cheese or feed samples. Analyses of the elements were carried out by inductively coupled plasma-optical emission spectrophotometer (*PerkinElmer Elan DRC II)* and inductively coupled plasma mass spectrometer (*Perkin Elmer Optima 2000 DV)*. At lower levels of contamination, ICP-MS provided lower detection limits for measurement (As, Cd, Cr, Cu, Mn, V, Ni and Pb), while ICP-OES was used for measuring higher concentrations of metals such as Fe and Zn. The instrumental conditions and operating variables of the instruments are summarized in *Table 3*.

Table 3: Variables and conditions of ICP-MS and ICP-OES

PerkinElmer Optima 2000™ DV	
RF Power	1450 W
Plasma Flow	15 L/min
Nebulizer Flow	0.65 L/min
Auxilliary Flow	0.5 L/min
Sample Pump Flow	1.5 mL/min
Neb. GemCone™	Low-flow
Nebulizer Chamber	Cyclonic
Injector	Alumina, 2 mm
Processing Mode	Area
PerkinElmer Optima 2000™ DV	
RF Power	1550 W
Plasma Gas	15 L/min
Nebulizer Gas	0.54 L/min
Auxilliary Gas	0.975 L/min
Sample Uptake	0.7 mL/min
Nebulizer	Meinhard Type K3
Sampler and Skinner	Platinum
Injector	Alumina, 0.85 mm
Reaction Gas	NH_3

Statistical analysis

All statistical analyses were carried out with the computer software SPSS Statistics 17.0. The data were statistically analyzed using one-way analysis of variance (ANOVA) and *t*-test to examine statistical significance of differences in the mean concentration of heavy metals obtained in milk and cheese samples. The levels were found significantly different at $P < 0.05$.

Results and Discussion

Metal concentrations in milk samples

Ten heavy metals were detected in the cow milk: As, Cd, Cr, Cu, Fe, Mn, V, Ni, Pb and Zn. Total concentrations of elements in the 40 samples analysed (20 from highway, and 20 from non-polluted green areas) are shown in *Table 4*. The content of lead, copper, vanadium, arsenic in milk collected from traffic intensive areas was significantly high, almost twice to three times as much as in that collected from green rural areas. Lower, but significant difference was detected in the concentrations of iron, however, milk from highway areas showed similar Ni concentrations to those observed in milk collected from non-polluted green areas. The contents of Zn reached the highest value of all the elements tested in both groups. In cow milk, about the same concentrations of this element have previously been observed by some authors [7]. The higher concentrations of zinc in milk results mainly from metal pesticides according to Watson [1], or zinc can be transferred from machines and tools used in milk collection procedure. No cadmium pollution could be detected in the milk from the green areas, however, 5.18 $\mu g\ kg^{-1}$ Cd content was measured in the milk of highway areas.

In the milk collected from the highway areas, the concentrations of vanadium were measured three times higher than in the milk from non-polluted green areas. The concentrations of Cr and Fe detected in this study were quite similar to those measured under similar conditions in Poland [9], however As and Pb contents were detected higher in our study.

Metal concentrations in cheese samples

The concentrations of metal elements measured in semi-hard cheese (*Trappista*) are shown in *Table 5*. The sequence of heavy metals in the cheeses made from milk collected in non-polluted green or highway areas were as follows: As, Cd < V < Pb < Cr < Mn <Cu<Ni< Fe < Zn, and As, Cd < V < Pb < Mn < Cr < Cu < Ni < Fe < Zn, respectively.

Arsenic and cadmium concentrations in both groups of cheese samples were below detection limits. Chrome, copper, iron, and lead showed significantly higher concentrations in the cheese from highway areas, than in the cheese from non-polluted green areas. The treatment and the process of dairy production as well as the equipment used can influence the element concentrations [13]. The range of other trace elements, Ni and Fe were found similar in both cheese samples. The highest amount of V was detected in cheese made of milk collected from highway areas (119.9 $\mu g\ kg^{-1}$), the level of this element was significantly lower (75.3 $\mu g\ kg^{-1}$) than that of the cheese from green area. Among all the elements detected in this study Zn had the highest concentration in both treatments. High level of zinc and iron can be also explained by manufacturing processes.

The mean concentrations of heavy metals obtained for cheese samples in this study were similar, however Cr level was higher than those published in other literature [2, 3].

Table 4: Heavy metal contents ($\mu g\ kg^{-1}$) of cow milk from lactating cows kept in non-polluted green or highway conditions (n=20 in each area)

Elements	Non-polluted green area		Highway area		P-value (p<0.05)
	Mean	SD*	Mean	SD*	
Cr	14.7	2.2	18.3	0.9	0.04
Cu	137.1	22.7	336.2	7.9	<0.01
Fe	778.0	145.6	797.2	83.1	0.03
V	30.1	4.5	90.8	10.0	<0.01
Zn	2240.5	517.4	1493.7	124.5	0.01
Mn	25.8	9.7	16.9	2.0	0.01
Cd	ND*	-	5.2	1.6	0.02
As	23.3	7.8	52.1	5.2	<0.01
Pb	11.7	5.1	24.9	12.4	0.05
Ni	24.1	3.0	25.5	2.5	0.08

ND: not detectable
SD: standard deviation

Table 5: Heavy metal contents (μg kg^{-1}) of cheese (*Trappista*) made of milk from lactating cows kept in non-polluted green or highway conditions (n=20 in each area)

Elements	Non-polluted green area		Highway area		P-value (p<0.05)
	Mean	SD*	Mean	SD*	
Cr	314.1	93.9	528.5	141.2	0.05
Cu	455.5	77.9	695.4	62.2	<0.01
Fe	7258.0	45.7	7979.3	63.6	0.03
V	75.3	22.3	119.9	49.4	0.09
Zn	23890.6	1783.5	19246.5	2066.6	<0.01
Mn	366.6	74.2	417.9	45.1	0.09
Cd	ND*	-	ND*	-	-
As	ND*	-	ND*	-	-
Pb	125.7	51.0	148.9	42.4	0.05
Ni	929.5	213.6	902.7	149.7	0.13

ND: not detectable
SD: standard deviation

Conclusion

Differences in the concentrations of major and minor chemical elements in milk depend on many circumstances which are related to geographical location, soil type, nutrition, breed, age, lactation period, geographical location, mineral status of animals. However, certain heavy metal concentrations in milk from polluted areas are high compared to milk from non-polluted areas. These differences can be detected even in milk products such as cheese. Our results confirm that attention must be paid to circumstances entailing continuous emission of heavy metal elements into the environment. The method of technology and quality control of milk and dairy product processes should be monitored regarding certain toxic elements in both fresh milk and milk products.

ACKNOWLEDGEMENT

We acknowledge the financial support of this work by the Hungarian State and the European Union under the TAMOP-4.2.1/B-09/1/KONV-2010-0003 project.

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