Lead and cadmium levels in raw cow's milk from an industrialised Croatian region determined by electrothermal atomic absorption spectrometry

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ABSTRACT: A simple and accurate method for lead and cadmium determination in raw cow's milk by electrothermal atomic absorption spectrometry (ET-AAS) is described. Milk from fifteen farms near Križ in Zagreb region was sampled from the collective sample directly into plastic bottles (to avoid contamination) four times during the March of 2000. The milk samples were digested in an MLS-1200 Mega Microwave digestion system with MDR Technology. Lead and cadmium were determined directly by ET-AAS in the solutions of digested samples. Statistical analyses were performed using statistical software SAS v. 8.0. Both the farm and the date as well as their interaction ($P < 0.0001$) had a statistically significant influence on Pb and Cd levels in cow's milk. Pb (0.27 ± 0.06 mg/kg DM) and Cd (0.037 ± 0.007 mg/g DM) contents were not correlated ($R = 0.11$) and were lower in all examined samples than the tolerance limit defined by Croatian regulations (Pb < 100 μg/l and Cd < 10 μg/l). Chemical and statistical analyses showed that differences between the farms were not due to feed. This implies that in order to avoid milk contamination by toxic trace elements great care of stable microclimate and all apparatuses and dishes in contact with milk should be taken.

Keywords: lead; cadmium; raw cow's milk; electrothermal atomic absorption spectrometry

Milk and dairy products are important components of human food. The presence of toxic metals in the food chain is the result of environmental pollution and their concentrations need to be controlled constantly. Therefore analytical methods should be not only as accurate as possible but also as simple and as fast as possible while maintaining required accuracy.

Two ubiquitous harmful metals are lead and cadmium. The content of lead and cadmium in milk and dairy products is usually very low, except when animals have consumed contaminated feed. In the area of the Meža River in Slovenia, a high level of lead was found in vegetables after ten years since a lead-melting furnace was shut down (Blanuša *et al*., 1990).

Electrothermal and flame atomic absorption spectrometry are the most frequently and widely used techniques for the determination of metal contents in milk (Cerkvenik *et al*., 2000), dairy products (Cabrera *et al*., 1995) and in other kinds of foods (Jorhem, 1993). In investigations of lead and cadmium levels in raw cow's milk from six regions in Slovenia (Cerkvenik *et al*., 2000) and on 111 dairy farms in 15 counties in California (Bruhn and Franke, 1976), lead and cadmium were determined by flame atomic absorption spectrometry (FA-AAS) as a diethylammonium diethyldithiocarbamate complex in methyl isobutyl ketone (Cerkvenik *et al*., 2000) and as ammonium pyrrolidino carbo dithioate complexes in isoamylacetate (Bruhn and Franke, 1976). The determined Pb and Cd levels were found to correspond to the tolerance limit defined by Slovenian and USA regulations. In raw and pasteurised cow and goat milk lead and cadmium were also determined by FA-AAS (Lopez *et al*., 1985). Electrothermal atomic absorption spectrometry (ET-AAS) is another commonly used technique for determination of metal levels in raw milk (Jeng *et al*., 1994), pasteurised

milk (Barlow, 1977) and in dairy products (Cabrera *et al*., 1995). Samples of dairy products, in which lead and cadmium levels were determined, were prepared according to two different procedures: the slurry procedure and nitric acid mineralization in a Microwave Acid Digestion Bomb (Cabrera *et al*., 1995). The lead concentration in cow's milk from an unpolluted Croatian rural area, as determined by ET-AAS, was lower than those from an area with increased environmental lead exposure (Telišman *et al*., 1985a,b).

These results prompted us to determine the concentrations of lead and cadmium in raw cow's milk from a rural area near Zagreb**,** industrially most developed Croatian region. As farmers sell their milk to leading Croatian dairies, monitoring of Pb and Cd levels is important for ensuring a good quality of final product. In this paper a simple and reproducible method for determination of lead and cadmium by ET-AAS is described.

MATERIAL AND METHODS

Sampling strategy. The samples of raw cow's milk were collected four times during the March of 2000 from fifteen farm locations near Križ in Zagreb region. Lead and cadmium were analysed by ET-AAS in a total of 60 milk samples. The animals on each farm were fed dry hay and mineral premixes and had an access to the pump's water. The concentrations of lead and cadmium in raw milk are very low and possible contamination of samples is relatively high. Therefore milk is sampled from the collective sample of farms directly into carefully washed plastic bottles. The rest of milk samples was frozen.

Precautions against contamination. All used laboratory ware was cleaned with chromsulphuric acid and rinsed with deionised water. After that the laboratory ware was cleaned by soaking in 10% HNO_3 for 24 h, rinsed with deionised water, soaked in 3% EDTA solution for 24 h and again rinsed with deionised water.

Analytical procedure. 0.500 g of raw cow's milk is treated with 6 ml of 65% HNO_3 and 1 ml 30% H_2O_2, mixed and digested in a MLS-1200 Mega Microwave digestion system with MDR Technology, for elimination of the organic part of milk. After that lead and cadmium were directly determined by ET-AAS. All solutions were prepared with deionised water. Chemicals of the highest available purity were used. HNO_3 (Riedel) was used for the dilution and mineralization of raw milk samples. 30% H_2O_2, ρ = 1.11 g/l (Riedel) was also used for digestion. For the calibration standard lead and cadmium nitrate solutions (Merck, ρ = 1 g/l) were used.

Instrumentation. A Unicam 929 AA-Solar System ET-AAS equipped with Unicam GF-90 graphite furnace, an FS-90 autosampler and Canon BJ-2000 ex printer was used. The instrument was controlled by PC that processed the signals. Argon was used as a purge gas. For lead and cadmium quantifications, a simple aliquot volume of 20 µl was injected automatically into the graphite tube. Instrumental conditions for the determination of lead and cadmium in raw milk are given in Table 1.

Statistics. Data were analysed as 15 × 4 factorial design by General Linear Models procedure. Mean differences were separated by Duncan's Multiple Range Test. The level of significance was set at $P < 0.05$. All analyses were performed using statistical software SAS v. 8.0.

Table 1. Instrumental conditions for the determination of lead and cadmium in raw cow's milk by ET-AAS

	Lead	Cadmium
Primary wavelength	217.00 nm	228.80 nm
Band pass	0.5 nm	0.5 nm
Injection volume	20 µl	20 µl
Cuvette type	electrographite	electrographite
Maximum ash temperature	600°C	800°C
Atomisation temperature	1 400°C	1 200°C
Matrix modifier	50 µg/l lanthanum nitrate	50 µg/l lanthanum nitrate
Hollow cathode lamp current	10 mA	8 mA

RESULTS AND DISCUSSION

Lead and cadmium levels in raw cow's milk were determined by ET-AAS. The levels of Pb (0.27 ± 0.06 mg/kg DM) and Cd (0.037 ± 0.007 mg/kg DM) in feed did not differ significantly between farms and were not correlated. All examined milk samples showed the levels below 100 μg/l for lead and 10 μg/l for cadmium. These limits were established by Croatian legislation (Narodne Novine, 1994).

Table 2. ANOVA summary of the farm, date and their interaction effect on Pb and Cd levels in raw cow's milk. Means with same letter are not significantly different

	Pb	Cd
	Probability	
Farm	< 0.0001	< 0.0001
Date	< 0.0001	< 0.0001
Farm × date	< 0.0001	< 0.0001
	μg/l	
Main effects		
Farm		
I	19.93^{k}	3.03^{k}
II	57.96^{a}	5.45^{f}
III	31.12^{g}	6.26^{b}
IV	26.21^{j}	6.44^{a}
V	34.83^{e,f}	5.63^{e}
VI	35.81^{e}	5.64^{e}
VII	33.87^{f}	4.05^{j}
VIII	37.08^{d}	6.09^{c}
IX	39.40^{c}	5.61^{e}
X	27.32^{i,j}	5.78^{d}
XI	30.34^{g}	5.44^{f}
XII	28.67^{h}	5.12^{g}
XIII	28.16^{h,i}	4.48^{i}
XIV	49.28^{b}	4.88^{h}
XV	30.82^{g}	5.82^{d}
Date		
1. 3.	32.47^{c}	5.38^{b}
6. 3.	34.43^{b}	5.41^{a,b}
15. 3.	36.57^{a}	5.46^{a}
22. 3.	32.68^{c}	5.01^{c}

The results of factorial ANOVA are summarised in Table 2. Both farm and date had a statistically significant influence on Pb and Cd levels in cow's milk, as well as their interaction (Figure 1).

The highest Pb level was found in milk from farm II (57.96 μg/l) and the lowest in milk from farm I (19.93 μg/l). For a majority of farms, 8, Pb level was in the range 28–36 μg/l. Earlier studies (Telišman *et al*., 1985a,b) showed that Pb levels in cow's milk from lead-contaminated areas in Croatia were in the range 2.1–82.7 μg/l with median value 14.9 μg/l, while those in the control group were in the range 1.2–8.2 μg/l with median value 3.1 μg/l. These results imply that although Pb levels were below the tolerance limit on all 15 farms, this region is exposed to lead contamination and Pb levels should be monitored in order to ensure milk safety. In 1994–1998 Pb levels in 98% of samples in Slovenia were below the detectable limit. The highest detected Pb concentration (Cerkvenik *et al*., 2000) was higher than that detected in this study. Higher Pb levels were also detected in California (Bruhn and Franke, 1976). The highest Pb levels were recorded on 15. 3. Levels recorded on 1. 3. and 22. 3. were the lowest and significantly lower than the levels recorded on 15. 3. and 6. 3.

The highest Cd level was found in milk from farm IV (6.44 μg/l), the lowest in milk from farm I (3.03 μg/l) and for a majority of farms, 6, Cd level was in the range 5.0–6.0 μg/l. The results are similar to those obtained in the study of Californian farms where mean Cd concentration was 6.0 μg/kg (Bruhn and Franke, 1976).

Similarly like for Pb, the highest Cd levels were recorded on 15. 3. but they are not significantly different from the levels recorded on 6. 3. which are not significantly different from the levels recorded on 1. 3. Cd levels recorded on 22. 3. were lowest, and significantly different from the levels recorded on the other dates.

Differences between farms are not due to feed as shown by chemical and statistical analyses. The results imply that in order to avoid milk contamination by toxic trace elements great care of stable microclimate and all apparatuses and dishes in contact with milk should be taken.

The correlation analysis showed that there was no correlation between Pb and Cd levels in cow's milk on the investigated farms (R = 0.11).

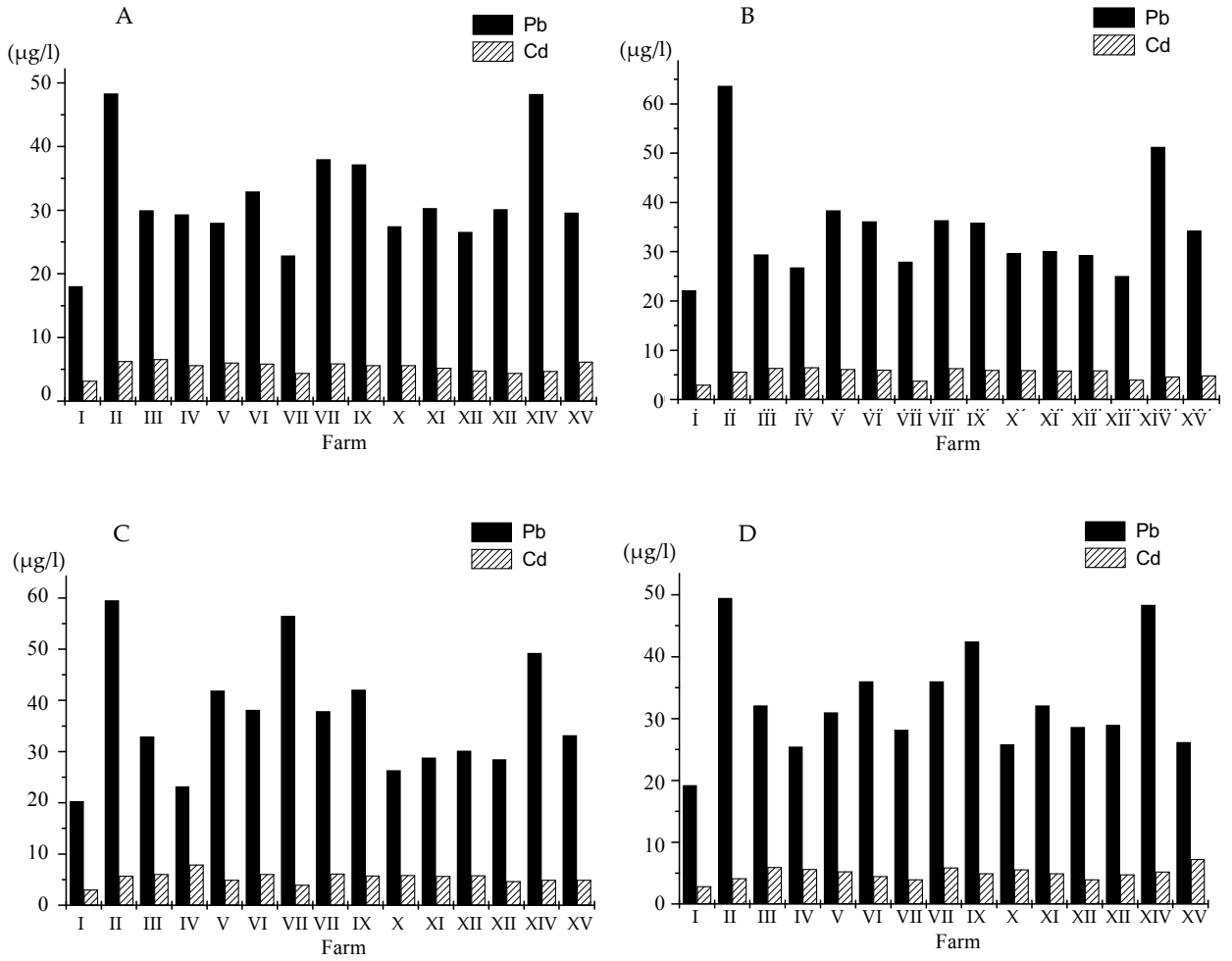

Figure 1. Pb and Cd levels in raw cow's milk from investigated farms on A) 1. 3., B) 6. 3., C) 15. 3. and D) 22. 3.

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ABSTRAKT

Hladiny olova a kadmia v syrovém kravském mléce z průmyslově rozvinuté oblasti Chorvatska zjištěné elektrotermickou atomovou absorpční spektrometrií

V práci je uveden popis jednoduché a přesné metody pro stanovení olova a kadmia v syrovém kravském mléce pomocí elektrotermické atomové absorpční spektrometrie (ET-AAS). Čtyřikrát během měsíce března 2000 byly odebrány vzorky mléka z 15 farem v okolí Križe v záhřebské oblasti z cisternového vzorku přímo do plastových lahví (aby se předešlo kontaminaci). Digesce těchto vzorků mléka se uskutečnila v mikrovlnném digesčním systému MLS-1200 Mega s technologií MDR. Olovo a kadmium bylo stanoveno přímo pomocí ET-AAS v roztocích mineralizovaných vzorků. Ke statistickým analýzám byl použit statistický software SAS v. 8.0. Statisticky významný vliv na hladiny Pb a Cd v kravském mléce měla farma i termín odběru, jakož i jejich interakce ($P < 0,0001$). Nebyla zjištěna žádná korelace mezi obsahem Pb (0,27 ± 0,06 mg/kg sušiny) a Cd (0,037 ± 0,007 mg/kg sušiny) ($R = 0,11$), a tyto obsahy byly ve všech zkoumaných vzorcích nižší, než je přípustná mezní hodnota stanovená chorvatskou legislativou (Pb < 100 μg/l a Cd < 10 μg/l). Chemické a statistické analýzy naznačily, že rozdíly mezi farmami nezpůsobilo krmivo. Aby se zabránilo kontaminaci mléka toxickými stopovými prvky, je třeba věnovat velkou pozornost stájovému mikroklimatu a veškerému zařízení a nádobám, které přicházejí do styku s mlékem.

Klíčová slova: olovo; kadmium; syrové kravské mléko; elektrotermická atomová absorpční spektrometrie

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