International Food Research Journal 19 (3): 1089-1094 (2012)

Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine

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Abstract: Heavy metal contents in 160 samples of commercially available cow's milk (powder and liquid), dairy products (yoghurt, labaneh and white cheese) and hen' eggs (conventional farms and courtyard farms) were investigated using GAAS. Mean concentrations of metals (µg/g) in milk and dairy samples analyzed ranged between 0.022-0.057 for Cd, ND-0.93 for Pb, 0.62-0.85 for Cu and 3.2-12.91 for Fe. Results indicated high concentrations of Pb and Cd especially in powder milk samples. The lowest concentrations of metals were found in white cheese followed by liquid milk. Mean metal concentrations (µg/g) in hen's eggs were 0.021-0.049 for Cd, 0.06-0.48 for Pb, 2.29-3.26 for Cu and 15.25-33.52 for Fe. Hen's eggs were found to contain significantly more Fe and Cu than milk or dairy products but generally less Pb and Cd. Finally, further investigations are needed to identify the cause of elevated Pb and Cd levels especially in milk and dairy products.

Keywords: Heavy metals, milk, dairy products, hen's eggs, Palestine

Introduction

Heavy metals are persistent contaminants in the environment that can cause serious environmental and health hazards. They are released into the environment from natural as well as man-made activities. Some heavy metals (like Cu and Fe) are essential to maintain proper metabolic activity in living organisms; others (like Pb and Cd) are non-essential and have no biological role (Ayar *et al*., 2009; Qin *et al*., 2009). However, at high concentrations, they can cause toxicity to living organisms (Li *et al*, 2005).

Milk is a complex, bioactive substance that promotes growth and development of mammalian infants. It is considered as a nearly complete food since it is a good source of proteins, fats, sugars vitamins and minerals. Therefore, milk and dairy products are important components of human diets that are widely consumed by human children and adults especially elderly people around the World (Buldini *et al*., 2002; Enb *et al*., 2009; Qin *et al*., 2009). Although, milk is an ideal source of macroelement (Ca, K and P) and microelemts (Cu, Fe, Zn, Se), addition amounts of contaminant metals might enter milk and dairy products reaching levels that are harmful to humans (Qin *et al*., 2009). Milk and dairy products become contaminated with heavy metals either through food stuff and water or through manufacturing and packaging processes (Anastasio *et al*., 2006; Ayar *et al*., 2009). Bakircioglu *et al.* (2011) investigated the concentrations of Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn in cheese samples packaged in plastic and tin containers. They found that there were considerable differences among of the studied element contents of cheese samples packaged in tin and plastic containers. They concluded that, cheese types and packaging materials play a key role in the content of trace metal.

Hen's eggs are considered as a highly-nutritious food that is of great importance for human health (Surai and Sparks, 2001). However, eggs might contain elevated levels of heavy metals that originate mainly from food and water. Knowledge of the mineral content of eggs is becoming increasingly important for many reasons that are related to health and nutritional value of eggs, the consequences of egg metals on its embryonic development and the use of eggs as bioindicators for environmental metal pollution (Surai and Sparks, 2001; Sparks, 2006; Pappas *et al*., 2006). Altogether, milk, dairy products and chicken eggs constitute a major food source around the World. Therefore, monitoring heavy metal levels in milk, dairy products and chicken eggs is of great importance for nutritional, toxicological and environmental purposes.

In the West Bank, studies concerning heavy metal levels in milk, dairy products and chicken eggs are completely lacking, although these products are typical food stuff that is largely consumed in the in

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the Palestine. Therefore, The present study aimed at evaluating the heavy metal quality of cow's milk (liquid and dry), 3 dairy products (yoghurt, white cheese and labaneh) and hen's eggs (farms and organic) consumed in the West Bank. Levels of two essential metals (Cu and Fe) and two nonessential metals (Pb and Cd) were determined using atomic absorption spectrophotometer.

Materials and Methods

Sample collection

A total of 160 samples of commercially available cow's milk (liquid and dry), dairy products (yoghurt, white cheese, and labaneh) and hen's eggs (from conventional farms and small scale courtyard farms) were purchased from supermarkets in Ramallah City in the West Bank. Samples were belonging to 21 different brands 14 of which were local and the remaining 7 were foreign companies. Sample collection took place twice during 2009. The name of each brand was substituted by an alphabet from A-U. Names were followed by either L meaning the product belongs to a local company, or F meaning that the product belongs to a foreign company.

Sample analysis

Samples of different dairy products were kept in their original packages and transferred to the lab in an ice box. Subsamples of from each products were oven-dreid at 60°C till constant weights were reached. Thereafter, dry samples were ground to powder using a grinder with stainless steel knife, then stored in clean glass vials for later analysis.

Duplicate sub-samples (about 0.2 g fine powder) from each product were digested in a mixture of 1:1 nitric: perchloric acids (Suprapur, Merck) using a heating block (Swaileh *et al.* 2009). At the end of digestion, mixture volumes were adjusted to 10 ml using double distilled water. Blanks and reference material (Skim Milk Powder, BCR®-151 provided by the European Commission, Joint Research Centre-Institute for Reference Materials and Measurements, Brussels, Belgium) were run with the samples. Finally, concentrations of Cu, Cd, Pb and Fe were measured by a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer, AAnalyst 600). The pyrolysis-atomization temperatures used were 500-1500, 1200-2000, 1400-2100, and 850-1600 °C for Cd, Cu, Fe and Pb, respectively. All accepted recoveries were between 97.5% and 101% (Table 1).

Statistical analysis

Data were analysed using Statistix 9.0 software (Analytical Software, Tallahassee, FL, 2008).

Table 1. Mean certified metal concentration (μg/g dry weight) in the reference material (Skim Milk Powder, BCR®-151) used in the present study, mean obtained values and percentage recovery obtained for each metal measured

	Certified value	Mean obtained value	Average %recovery
Cu	5.23±0.08	5.219	99.8
Cd	101±8.0*	98.98*	98
Pb	2.002±0.026	2.022	101
Fe	50.1±1.30	48.85	97.5

*: ng/g

Table 2. Concentrations of metals (Means ± SE) in powder and liquid milk consumed in the West Bank, Palestine. Minimum and maximum values are shown between brackets. Samples were collected during 2009 and 4 samples were analyzed/each brand. F: Foreign, L: Local.

Product/Brand	Cd (ng/g)	Cu (μg/g)	Pb (μg/g)	Fe (μg/g)
Powder Milk				
A-F	33.0^b±16.13 (4-78)	0.66±0.02 (0.61-0.72)	1.01±0.11 (0.75-1.20)	5.32^b±1.19 (1.75-6.80)
B-F	38.7^b±8.78 (17-60)	0.68±0.03 (0.61-0.74)	0.78±0.03 (0.75-0.86)	46.62^a±1.46 (43.9-49.3)
C-F	26.0^b±0.41 (25-27)	0.67±0.05 (0.57-0.79)	0.86±0.12 (0.73-1.21)	5.02^b±1.88 (1.17-10.10)
D-F	117.2^a±40.47 (28-224)	0.65±0.04 (0.55-0.77)	1.08±0.08 (0.95-1.32)	5.57^b±1.43 (1.60-8.40)
E-F	ND	0.68±0.02 (0.64-0.75)	0.99±0.09 (0.78-1.20)	8.17^b±2.04 (4.60-13.90)
F-F	ND	0.65±0.03 (0.60-0.72)	0.89±0.08 (0.72-1.04)	6.75^b±1.49 (3.70-9.60)
p-value	*0.0434*	*0.9820*	*0.2559*	*0.0001*
Liquid Milk				
G-L	102.0^a±33.33 (24-172)	0.64±0.05 (0.49-0.73)	0.17±0.01 (0.16-0.21)	7.20±2.30 (1.10-11.90)
H-L	27.75^b±1.03 (25-30)	0.66±0.07 (0.55-0.86)	0.27±0.12 (0.10-0.61)	5.62±0.47 (4.60-6.60)
I-L	ND	0.63±0.09 (0.51-0.88)	0.20±0.02 (0.17-0.23)	11.02±4.74 (3.50-24.90)
J-L	5.75^b±1.84 (1-10)	0.60±0.02 (0.57-0.64)	0.18±0.01 (0.15-0.22)	6.60±0.18 (6.2-7.00)
K-L	31.00^b±3.67 (22-40)	0.69±0.11 (0.42-0.96)	0.23±0.09 (0.07-0.49)	8.20±0.79 (6.40-10.00)
L-L	22.75^b±3.06 (15-30)	0.57±0.03 (0.52-0.66)	0.18±0.01 (0.15-0.21)	10.00±0.69 (8.10-11.40)
M-F	25.00^b±9.97 (8-53)	0.57±0.04 (0.46-0.67)	0.13±0.04 (0.07-0.23)	8.95±2.39 (4.30-13.70)
p-value	*0.0031*	*0.8363*	*0.7868*	*0.6263*

Values with different letters within a column are significantly different at $p \le 0.05$

Statistical differences between the same product from different brands were tested using one way ANOVA. Where significant differences were observed, Tukey HSD paiwise comparison test was applied. Differences were considered significant at p values ≤ 0.05.

Results and Discussion

Metal contents of cow's milk, dairy products and hen's eggs are summarized in Tables 2-5. In all samples analyzed, concentrations of Fe were the highest and those of Cd were the lowest. Table 2 shows metal concentrations in milk powder from 6 foreign brands. Cd and Fe levels indicated significant differences between brands however; Cu and Pb levels were similar in powder milk from the 6 brands. Mean

Table 3. Concentrations of metals (Means ± SE) in dairy products (Yoghurt and Labaneh) consumed in the West Bank, Palestine. Minimum and maximum values are shown between brackets. Samples were collected during 2009 and 4 samples were analyzed/each brand. F: Foreign, L: Local.

Product/Brand	Cd (ng/g)	Cu (µg/g)	Pb (µg/g)	Fe (µg/g)
Yoghurt				
G-L	39.50±11.38	0.75^{b}±0.08	0.13±0.05	7.97±1.00
	(17-65)	(0.63-0.95)	(0.04-0.27)	(5.8-10.3)
N-L	63.25±20.17	1.34^{a}±0.16	0.04±0.01	20.10±10.52
	(8-103)	(1.01-1.76)	(0.03-0.05)	(2.7-47.7)
I-L	53.25±11.24	0.96^{ab}±0.18	0.34±0.12	11.82±0.54
	(25-80)	(0.5-1.37)	(0.11-0.69)	(10.8-12.9)
KB-L	47.25±5.95	0.73^{b}±0.04	0.21±0.10	5.60±0.59
	(37-65)	(0.63-0.81)	(0.05-0.49)	(4.10-7.0)
L-L	59.75±18.58	0.58^{b}±0.03	0.51±0.19	11.57±1.01
	(14-105)	(0.50-0.63)	(0.23-1.07)	(8.8-13.3)
O-L	69.50±0.29	0.62^{b}±0.04	0.3±0.05	8.12±1.81
	(69-70)	(0.52-0.74)	(0.09-10.00)	(3.3-11.9)
M-F	70.00±17.30	0.57^{b}±0.08	0.14±0.09	4.55±0.99
	(39-118)	(0.40-0.73)	(0.03-0.40)	(2.0-6.8)
p-value	*0.6654*	*0.0001*	*0.2541*	*0.1880*
Labaneh				
H-L	18.00^{ab}±3.89	0.91±0.04	0.22^{b}±0.08	5.55±1.38
	(7-24)	(0.77-0.96)	(0.04-0.37)	(3.20-8.50)
G-L	18.25^{ab}±6.21	0.78±0.14	0.21^{b}±0.07	4.65±2.38
	(3-33)	(0.47-1.10)	(0.01-0.28)	(0.60-10.90)
I-L	19.00^{ab}±2.16	0.94±0.14	0.31^{b}±0.08	8.32±3.66
	(15-25)	(0.77-1.36)	(0.19-0.45)	(1.40-17.60)
K-L	12.25^{b}±3.82	0.89±0.25	0.34^{b}±0.10	6.05±1.09
	(3-21)	(0.46-1.50)	(0.05-0.54)	(2.90-7.80)
L-L	22.75^{ab}±4.53	0.83±0.18	0.38^{ab}±0.08	7.42±1.87
	(12-34)	(0.52-1.25)	(0.24-0.59)	(3.20-11.00)
O-L	22.75^{ab}±5.07	0.78±0.09	0.18^{b}±0.03	5.95±1.68
	(8-31)	(0.64-1.06)	(0.13-0.25)	(1.70-8.90)
P-L	41.25^{a}±8.37	0.63±0.08	0.14^{b}±0.05	5.07±1.04
	(29-65)	(0.49-0.79)	(0.03-0.29)	(3.00-7.20)
Q-L	23.75^{ab}±5.95	0.98±0.26	0.87^{a}±0.26	4.50±1.83
	(7-34)	(0.65-1.76)	(0.28-1.53)	(1.10-8.20
M-F	20.00^{ab}±2.27	0.87±0.14	0.28^{b}±0.01	6.32±2.87
	(14-25)	(0.47-1.06)	(0.26-0.30)	(0.90-13.60)
p-value	*0.0351*	*0.9002*	*0.0029*	*0.9392*

Values with different letters within a column are significantly different at $p \leq 0.05$

Table 4. Concentrations of metals (Means ± SE) in white cheese consumed in the West Bank, Palestine. Minimum and maximum values are shown between brackets. Samples were collected during 2009 and 4 samples were analyzed/each brand. L: Local.

Product/Brand	Cd (ng/g)	Cu (µg/g)	Pb (µg/g)	Fe (µg/g)
White Cheese				
H-L	9.50±1.04	0.70^{b}±0.11	ND	2.64^{ab}±0.29
	(7-12)	(0.53-1.02)		(1.88-3.21)
I-L	37.5±17.05	0.87^{ab}±0.03	ND	3.39^{a}±0.25
	(6-68)	(0.81-0.92)		(2.91-4.11)
R-L	16.67±11.17	0.73^{b}±0.10	ND	3.72^{a}±0.53
	(5-39)	(0.54-0.96)		(2.97-5.30)
K-L	55.25±29.63	1.23^{a}±0.13	ND	2.01^{b}±0.09
	(3-110)	(0.96-1.50)		(1.81-2.21)
L-L	29.00±13.85	0.69^{b}±0.07	ND	3.86^{a}±0.13
	(4-63)	(0.52-0.86)		(3.57-4.21)
O-L	52.75±30.29	0.78^{b}±0.03	ND	3.71^{a}±0.17
	(2-130)	0.71-0.83)		(3.28-4.10)
Q-L	7.75±1.93	0.61^{b}±0.06	ND	3.05^{ab}±0.15
	(4-13)	(0.48-0.74)		(2.81-3.40)
p-value	*0.4120*	*0.0011*	--	*0.0007*

Values with different letters within a column are significantly different at $p \leq 0.05$

Cd levels in powdered milk from one brand (D-F) was significantly higher those from other brands and mean Fe levels in powdered milk from one brand (B-F) was significantly higher than other brands. The mean levels of metals were: 12.91, 0.93, 0.66 µg/g and 53.7 ng/g for Fe, Pb, Cu and Cd, respectively. Metal concentrations had the following order: Fe>Pb> Cu>Cd. The high concentration of Fe is caused by the enrichment of powder milk with iron that is practiced by most companies. Lead concentrations in powder milk were found to exceed the maximum allowed limit for Pb in milk powder and cheese that was set as 0.2 µg/g (Ayar, 2009). However, Cd concentrations were below the maximum allowed limit of 0.5 µg/g Cd in powder milk (Ayar, 2009).

Metal concentrations in liquid milk from 7 commercial brands are summarized in Table 2. No statistically significant differences between liquid milk brands were observed for Cu, Pb and Fe. The only significant difference was observed for Cd concentrations, where liquid milk from one brand (G-L) contained significantly higher Cd content (102.0 ng/g) than other brands. The mean concentrations of metals in different liquid milk samples were: 8.23, 0.62, 0.20 µg/g and 35.71 ng/g for Fe, Cu, Pb and Cd respectively. Levels of metals in liquid milk were having the following order: Fe>Cu>Pb>Cd and were less than those measured in powder milk. However, mean Pb and Cd concentrations in liquid milk exceed the maximum allowed limits of 0.02 and 0.01 µg/g for Pb and Cd in liquid milk respectively (Ayar, 2009).

Concentrations of the four metals in yoghurt and labaneh (a form of drained yoghurt) are shown in Table 3. No statistical differences in Cd, Pb and Fe concentrations were observed for yoghurt from all brands. Significant differences were observed for Cu concentration where yoghurt from I-L company contained the highest level of Cu. According to their concentrations in yoghurt, metals were having the following order: Fe>Pb> Cu>Cd. Concentrations of metals in labaneh were generally less than those in yoghurt. This may be caused by the loss of some metals by the drainage process. Statistical differences were observed for Cd and Pb concentrations in labaneh from different brands. The order of metals was Fe>Cu> Pb>Cd.

White cheese is the main and traditional form of cheese made and consumed in the West Bank (CHF, 2011). Other types of cheese are less commonly consumed and are imported from neighboring countries like Turkey. Results of elemental white cheese analysis from 7 local brands are summarized in Table 4. Generally, cheese is characterized by low concentrations of metals except Cu. Compared to all products analyzed in the present study, white cheese contained the lowest concentrations of Fe and Pb (below detection limits) and the second lowest Cd concentration. With regard to Cu, white cheese comes second among dairy products after labaneh which was found to have the highest levels of Cu. As the two products undergo drainage during processing, this indicates that the drainage process causes a

Table 5. Concentrations of metals (Means ± SE) in Hen's eggs consumed in the West Bank, Palestine. Minimum and maximum values are shown between brackets. Samples were collected during 2009 and 4 samples were analyzed/each brand. L: Local.

Brand	Cd (ng/g)	Cu (µg/g)	Pb (µg/g)	Fe (µg/g)
S-L	49.5±25.11	3.26^{a}±0.47	0.48±0.30	21.55^{ab}±6.00
	(6-93)	(2.56-4.60)	(0.10-1.39)	(10.8-38.60)
T-L	21.42±2.60	2.56^{ab}±0.04	0.26±0.06	33.52^{a}±3.26
	(10-32)	(2.34-2.70)	(0.05-0.52)	(20.3-46.8)
U-L	46.75±27.71	2.29^{b}±0.19	0.06±0.02	15.25^{b}±3.65
	(5-125)	(1.83-2.70)	(0.02-0.11)	(8.7-25.7)
p-value	*0.4174*	*0.040*	*0.2021*	*0.0191*
Overall	35.67±9.70	2.70±0.14	0.27±0.08	25.96±3.01
	(5-125)	(1.83-4.60)	(0.02-1.39)	(8.7-46.8)

Values with different letters within a column are significantly different at p≤0.05

Table 6. Cow's milk, dairy products and Hen's eggs ranked according to their metal richness. TRS: Total Rank Score.

	Metal concentration rank score				
Product	Cd	Cu	Pb	Fe	TRS
P. Milk	5	2	6	5	18
L. Milk	4	1	2	3	10
Yoghurt	6	3	4	4	17
Labaneh	1	5	5	2	13
Cheese	2	4	1	1	8
Eggs	3	6	3	6	18

Table 7. Concentrations of metals (µg/g) in Cow's milk from different countries compared to those of the present study.

Study	Country/milk type	Cd	Pb	Cu	Fe
Enb *et al.* (2009)	Egypt/raw	0.086	0.066	0.124	0.682
Valiukenaite *et al.* (2006)	Lithuania/raw	0.004	0.005	--	--
Akpanyung (2006)	Nigeria/powder	--	--	6-15	118
Ayar *et al.* (2009)	Turkey/raw	0.017	0.103	--	--
	/powder	0.024	0.054	--	--
Qin *et al.* (2009)	China/commercial	0.004	0.035	0.17	2.21
	Japan/commercial	0.001	0.012	0.23	1.51
Farid *et al.* (2004)	S. Arabia/commercial	0.005	0.003	0.049	--
	/powder	0.003	0.002	0.037	--
Present study	Palestine/commercial	0.036	0.20	0.62	8.23
	/powder	0.054	0.93	0.66	12.91

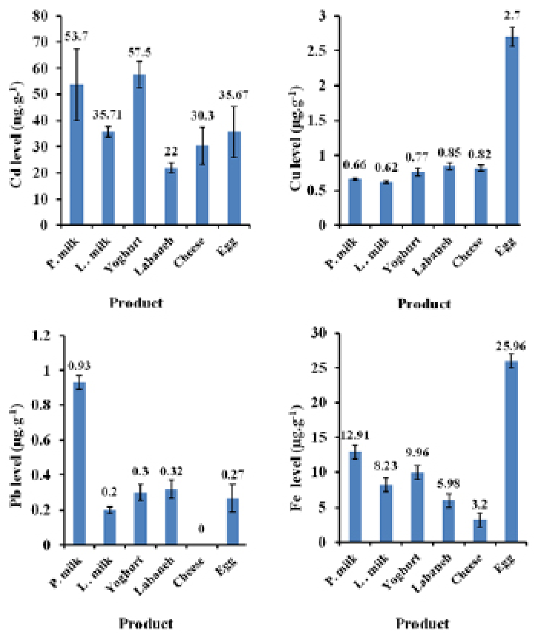

Figure 1. Overall mean (±SE) concentrations of metals in cow's milk, dairy products and hen's eggs consumed in the West Bank, Palestine.

significant decrease in Fe, Cd, and Pb concentrations but an increase in Cu levels.

Hen's eggs are considered as an important source of nutrients, including micro minerals (Surai and Sparks, 2001) and are consumed largely in the West Bank. Eggs were collected from conventional poultry farms (S-L and U-L) and from a courtyard small scale farm (T-L). Eggs were found to contain high levels of Fe and Cu (essential metals) and much less levels of Cd and Pb (non essential metals) (Table 5). Actually, levels of Fe and Cu in eggs were significantly higher than those in milk or dairy products. Eggs from courtyard were containing significantly higher levels of Fe than those obtained from conventional farms. Metal concentrations in eggs were ordered as Fe>Cu>Pb>Cd. Fakayode and Olu-Owolabi (2003) studied levels of metals in chicken eggs from Nigeria. The overall average concentrations for Pb, Cd, Cu and Fe were 0.59, 0.07, 0.78, 23.2 µg/g, respectively. Khan and Naeem (2006) investigated elemental composition of Chicken eggs from Pakistan. The average concentrations of Pb, Cd, Cu and Fe ranged between 0.52-0.63, 0.07-0.08, 0.74-0.82 and 21.8-24.1 µg/g. These results are comparable to results of the present study.

Figure 1 shows the overall mean concentrations of the four metals in all samples analyzed for each product regardless of the brand name. The Figure indicates that eggs are of great nutritional value especially when it comes to the essential metals Fe and Cu. Powder milk contains the highest concentration of Pb and the second highest concentration of Cd among all products analyzed. Powder milk is an imported product that is not manufactured locally.

Ranking biological samples according to metal concentration gives a clear idea about metal richness (Swaileh *et al.*, 2009). When ranked according to metal concentrations (Table 6), eggs and milk powder were found to have the highest total rank score and cheese the lowest. The 6 products examined had the following decreasing order in total rank score powder milk, eggs>yoghurt> labaneh>liquid milk>cheese.

Milk is the raw material for all other dairy products and the final concentration of metals in any dairy product is affected by the concentration of metals in the milk used and the industrial processing. Table 7 shows a comparison between milk metal concentrations in the present study and those reported from other countries. The results indicate that milk in the present study contains higher levels of metals than most other countries. This is obvious especially in the case of Pb. Although Palestine is not an industrialized country, leaded fuel is still largely used which might be one reason for the high Pb levels recorded. In addition, processing and packaging of milk and dairy products may lead to an elevation in metal concentrations.

Conclusions

Concentrations of Cd, Pb, Cu and Fe in milk, dairy products and eggs consumed in the West Bank showed little variability with brand. Generally, Pb and Cd concentrations in milk and dairy products (except white cheese) exceeded the maximum allowed values. The elevated levels could be related to contamination during industry processing and environmental pollution. Powder milk was found to be the richest in metals while white cheese was found to be the poorest. Among the four metals studied, Fe concentrations were always the highest and Cd concentrations were always the lowest. Hen's eggs were found to have obviously higher concentrations of Fe and Cu than milk or dairy products. This emphasizes its nutritional value as a source of essential elements. No clear differences were observed between metals in eggs from conventional farms and courtyard farms. Comparing results of the present study with those of other studies revealed higher levels of metals, especially Pb, in milk and dairy products investigated in the present study. Results of hen's eggs analysis are comparable to those reported from other countries. Finally, the elevated levels of heavy metals (Pb and Cd) need further investigations to identify the cause of these elevated levels.

Acknowledgement

The authors would like to thank Birzeit University for the financial support of the project.

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