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Research paper

Determination of some metals in the commonly consumed dairy products randomly collected from the market in Alexandria - Egypt, with an emphasis on toxicity, permissible limits and risk assessment

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Abstract

The aim of this research was to evaluate the possible contamination by heavy metals of 30 random samples of raw milk and four other dairy products (pasteurized milk, white cheese, yellow cheese, and yoghurt), that were purchased from the three different regions in Alexandria, Egypt namely; (Eastern, Central and Western Alexandria). Each sample was homogenized, powdered, and mineralized in a microwave oven. Quantitative analyses of Al, Se, Zn, Cd, Cu and Pb were performed using an inductively coupled plasma-mass (ICP-MS) spectrometry. Western Alexandria samples had the highest recorded levels in Pb, Cd and Cu (7.421 ppm, and 0.673 ppm, and 5.013 ppm) consecutively. Highest levels detected for Al and Se were detected in samples collected from Eastern Alexandria (2.74 ppm and 0.093 ppm) successively. Zn (31.64 ppm) showed the highest concentration in a sample purchased from Central Alexandria. Further investigations of the levels of metals in bigger number of milk samples from different zones of Alexandria, Egypt are necessary, both to examine this problem from the toxicological, clinical, and epidemiological point of view and, to assess the exposure risk.

Keywords: *Toxicity; ICP-MS; Heavy Metals; Milk; Dairy Products ; Risk Assessment; Egypt.*

1. Introduction

The existence of heavy metals in the environment is considered as a major concern because of their toxicity and threat to human life as well as environment. (Katzi TG et al. 2009, Bilandzic N et al. 2011) Dairy products provide a great sense of eating pleasure due to their flavor and nice taste. Milk and dairy products are considered important foods regarding their protein and mineral contents essential for promoting the growth and maintenance of human life. (Katzi TG et al. 2009) Trace elements in foodstuffs are of significant importance because of their essential or toxic effect. Milk is known as an excellent source of Ca, and it can supply moderate amounts of Mg, smaller quantity of Zn and very small contents of Fe and Cu. (Bilandzic N et al. 2011, Rahimi E 2013) On the other hand, due to the developing environment pollution induced by industrial, and agricultural pollutions; milk and dairy products contain different amounts of toxic contaminants. (Ataro A et al. 2008, Karimi H et al. 2008, Bilandzic N et al. 2011) Consequently, it is also mandatory to determine and monitor the levels of these toxic metals (TMs) in milk and dairy products, because they are for the most part consumed by infants and children and, can significantly influence their health. The determination of TMs level in milk is particularly monitored by international organizations. (Steijns JM 2001, Licata P et al. 2004) Many reports indicated the presence of heavy metals in milk and other food products. (Caggiano R et al. 2005, Tuzen M et al. 2008)

Cu, Zn and Se are essential metals for normal function of our body, however, from the toxicological point of view, their intake in higher amounts above safe recommended levels may be risky to the human health. (Licata P et al. 2012) It is well-known that the Cd and Pb have the greatest injurious effects on renal tissue and nervous system, respectively. (Neal AP and Guilarte TR 2013) Contamination of dairy products with Aluminum may result from the environment, but the concentration of this element increases notably with processing, packaging and food additives. (Damond J 2005) There is a strong connection between Aluminum and Alzheimer's syndrome. Besides Alzheimer's, toxic levels of Aluminum has also been associated with Parkinson's disease. Chronic Aluminum exposure has contributed directly to hepatic failure and dementia. (Canadian Study of Health and Aging 2002) The determination of trace inorganic constituents in milk and dairy products is not an easy task due to their complex emulsion like matrices and low concentration levels of the metal ions. Most proposed procedures involve a step of digestion to eliminate the organic matrixes, which may involve several steps and contamination can become a serious obstacle for obtaining accurate data of trace quantities of elements. (Tuzen M and Soylak M 2007, Karimi H et al. 2008) Several analytical methods have been reported for the determination of trace metals in milk and dairy products including: inductively coupled plasma-mass (ICP-MS) spectrometry(Licata P et al.

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2004) or, atomic absorption spectroscopy (AAS).(Freschi GP et al. 2012) Knowing the cumulative effect of heavy metals and, from the toxicological and, dietary points of view, it is essential to assign the amount and absorption of heavy metals in milk and its products and compare the results with the permissible international limits, to be able to assess risk of exposure. Hegart PV in 2008, stated that in order to evaluate risk assessment through dietary exposure to metals, many parameters have to be estimated, namely; provisional tolerable daily intake (PTDI), provisional tolerable weakly intake (PTWI) and provisional tolerable monthly intake (PTMI).

2. Aim of the work

To evaluate the levels of some metal content among different dairy products collected from the open market in Alexandria, Egypt. Also, to compare the results with international standards regarding the permissible limits; and assess exposure to heavy metals due to the consumption of milk and other dairy products.

3. Material and methods

30 random samples of raw milk (RM), and four other dairy products {pasteurized milk (PM), white cheese (WC), yellow cheese(YC), and yoghurt(Y)} were randomly purchased from supermarkets in the three different regions in Alexandria, Egypt namely; (Eastern, Central and Western Alexandria). Sample preparation and analysis will be conducted; the samples will then be analyzed for some metals content (Al, Se, Zn, Cd, Cu and Pb) using inductively coupled plasma-mass (ICP-MS) spectrometry according to the procedure described by Jalbani N. et al. in 2009. The mean concentrations of the metals will be compared with the international permissible limits. Risk assessment by dietary exposure to metals through milk and dairy products consumption will be studied.

3.1. Chemicals

All the reagents and chemicals Merck (Darmstadt, Germany) were used of analytical grade. De-ionized water was used throughout the work. Concentrated nitric acid (65%), were spectroscopic grades Merck (Darmstadt, Germany).

3.2. Apparatus

Inductively Coupled Plasma Mass Spectrometry (ICP-MS), after microwave acid digestion was used in measurements of studied metals.

3.3. Sampling and analysis

30 random samples of raw milk and four other dairy products (pasteurized milk, white cheese, yellow cheese, and yoghurt) were purchased from supermarkets in the three different regions in Alexandria, Egypt namely; (Eastern, Central and Western Alexandria) in Alexandria city, Egypt during the year 2015. Samples were conditioned in sterile plastic container and kept at 4˚C until analyses that were carried out in same day.

3.4. Sample preparation

Six elements (Al, Se, Zn, Cd, Cu and Pb) were determined in each dairy product samples. Prior to analysis samples must be decomposed with the appropriate methods procedure with digest containing reduced amounts of carbon residues. In order to minimize the effects of the organic matrix, closed vessel acid decomposition in microwave oven system was used. Moreover, it may provide faster, more efficient process and reduce or eliminate the risk of sample contamination and losses of analytes. A microwave assisted-acid digestion procedure was carried out, in order to achieve a shorter digestion time and using minimum amount of acid. Duplicate of 2.0 mL of each dairy product samples were taken into microwave vessels but well shaken, opened and sampled by pouring directly from the original container into the microwave vessel. Added the samples to each vessels and 10 mL of a concentrated HNO3-H2O2 (2:1, v/v) and kept samples for 10 min at room temperature till the samples were homogenized, and then placed the vessels in covered PTFE container. This was then heated following a one-stage digestion programmed at 80% of total power (900 W), for 3 - 5 min. After cooling, the resulting solutions were evaporated to semidried mass to remove an excess acid, and then diluted up to 50.0 mL in volumetric flasks and kept as a stock sample solution, and were analysed using methodology given by Jalbani N. et al. in 2009. [17]

3.5. Statistical analysis

Statistical analysis was performed using SPSS statistical software (version 15; SPSS, Chicago, IL). Data were expressed as minimum, maximum and, mean ± standard deviations (SD).

4. Results

Comparison between Concentration ranges in mg/kg (ppm) of metals in different dairy products (n = 30 for each sampling site) purchased from Alexandria, Egypt were given in table (1). Western Alexandria samples had the highest recorded levels in Pb, Cd and Cu (7.421 ppm, and 0.673 ppm, and 5.013 ppm) consecutively. Highest levels detected for Al and Se were detected in samples collected from Eastern Alexandria (2.74 ppm and 0.093 ppm) successively. Zn showed the highest concentration in a sample purchased from Central Alexandria (31.64 ppm). The accepted daily intake (ADI) of Pb, Cd, Al, Cu, Zn and Se and mean concentration of these metals in all studied samples compared to their calculated daily intake from consumption of 200 ml milk or 200 g dairy product per day based on a caloric intake of 2,000 calories diet were presented in table (2). Pb had the highest calculated daily intake from consumption of 200 ml milk or 200 g dairy product per day (98.1%), while Al had the least calculated value (0.12%).

Table 1: Concentration Ranges in Mg/Kg (Ppm) of Metal in Milk, and Dairy Products Samples (N = 30 for Each Sampling Site)

Metal		Eastern Alexandria					Central Alexandria					Western Alexandria				
		RM	PM	WC	YC	Y	RM	PM	WC	YC	Y	RM	PM	WC	YC	Y
Al	Minimum	0.33	0.14	0.25	1.01	0.24	0.41	0.54	0.84	0.42	0.12	0.51	0.52	0.64	0.45	0.17
	Maximum	2.50	2.61	1.91	2.74	1.42	2.31	1.92	2.20	2.51	1.84	2.11	1.42	2.60	2.54	1.89
	Mean± SD	0.501 ± 0.107	1.324 ± 0.112	1.324 ± 0.112	0.403 ± 0.114	1.021 ± 0.121	0.320 ± 0.112	0.303 ± 0.121	1.221 ± 0.100	1.230 ± 0.152	0.306 ± 0.104	1.121 ± 0.111	1.420 ± 0.137	0.414 ± 0.141	1.111 ± 0.012	1.304 ± 0.152
Se	Minimum	0.010	0.012	0.005	0.005	0.011	0.002	0.003	0.004	0.002	0.001	0.015	0.015	0.006	0.011	0.015
	Maximum	0.080	0.029	0.025	0.093	0.019	0.021	0.040	0.011	0.035	0.062	0.010	0.045	0.050	0.042	0.034

	Mean± SD	0.016 ± 0.003	0.018 ± 0.002	0.014 ± 0.001	0.016 ± 0.003	0.016 ± 0.001	0.011 ± 0.010	0.012 ± 0.003	0.015 ± 0.002	0.013 ± 0.001	0.012 ± 0.003	0.018 ± 0.001	0.016 ± 0.001	0.012 ± 0.002	0.014 ± 0.002	0.016 ± 0.001
Zn	Minimum	1.740	1.621	2.300	2.690	2.650	1.610	1.631	2.311	2.601	2.621	1.742	1.541	2.420	2.890	2.545
	Maximum	7.414	15.84	31.60	14.30	14.04	6.421	12.84	31.64	15.30	12.07	7.114	15.04	21.56	17.70	22.04
	Mean± SD	4.770 ± 1.524	6.796 ± 3.740	10.75 ± 6.749	8.178 ± 4.108	5.520 ± 2.492	4.970 ± 1.312	7.701 ± 2.940	10.75 ± 6.749	8.178 ± 4.108	5.520 ± 2.492	4.570 ± 1.614	6.196 ± 3.241	8.75 ± 6.749	6.178 ± 4.118	9.520 ± 2.492
Cd	Minimum	0.004	0.034	0.052	0.085	0.110	0.014	0.054	0.071	0.085	0.110	0.007	0.039	0.072	0.035	0.112
	Maximum	0.516	0.672	0.618	0.290	0.598	0.321	0.611	0.662	0.290	0.598	0.516	0.673	0.614	0.210	0.538
	Mean± SD	0.288 ± 0.161	0.278 ± 0.186	0.200 ± 0.137	0.223 ± 0.056	0.239 ± 0.102	0.284 ± 0.158	0.268 ± 0.174	0.241 ± 0.167	0.232 ± 0.044	0.219 ± 0.110	0.256 ± 0.132	0.291 ± 0.184	0.217 ± 0.135	0.236 ± 0.156	0.215 ± 0.101
Cu	Minimum	1.437	0.091	0.722	0.235	0.016	1.414	0.093	0.715	0.232	0.014	1.415	0.087	0.385	0.215	0.016
	Maximum	5.002	2.439	2.592	2.037	1.640	5.010	2.421	2.162	2.018	1.672	5.013	2.414	3.590	4.032	1.940
	Mean± SD	2.836 ± 1.094	1.505 ± 0.703	1.379 ± 0.447	0.899 ± 0.451	0.623 ± 0.420	2.814 ± 1.107	1.512 ± 0.627	1.324 ± 0.487	0.864 ± 0.416	0.671 ± 0.411	2.732 ± 1.091	1.905 ± 0.752	1.364 ± 0.412	0.852 ± 0.418	0.663 ± 0.435
Pb	Minimum	0.018	0.742	0.298	1.600	0.270	0.019	0.727	0.273	1.635	0.270	0.019	0.642	0.271	1.920	0.259
	Maximum	6.754	5.805	5.416	7.015	5.400	7.054	6.015	5.926	7.011	5.381	5.724	5.254	7.421	7.011	5.353
	Mean± SD	1.850 ± 1.557	3.500 ± 1.517	2.900 ± 1.287	4.404 ± 1.607	3.053 ± 1.305	1.950 ± 1.335	3.540 ± 1.521	2.870 ± 1.247	3.904 ± 1.621	3.102 ± 1.105	1.812 ± 1.521	2.501 ± 1.350	2.840 ± 1.287	4.404 ± 1.607	3.051 ± 1.612

Table 2: The Accepted Daily Intake (ADI) of Pb, Cd, Al, Cu, Zn and Se and Mean Concentration of These Metals in All Studied Samples Compared to Their Calculated Daily Intake from Consumption of 200 Ml Milk or 200 G Dairy Product Per Day Based on A Caloric Intake of 2,000 Calories Diet.

Metals	ADI µg /70 kg person	Mean concentration of metals (ppm = µg/ml) in all examined samples	Calculated daily intake of metals from consumption of 200 ml milk or 200 g dairy product per day based on a caloric intake of 2,000 calories & calculated percentage of ADI.	
			µg /day / person	%
Lead (Pb)	252 [a]	3.541	247.87	98.01
Cadmium (Cd)	70 [a]	0.212	14.84	21.2
Aluminium (Al)	70,000 [a]	1.211	84.77	0.12
Copper	35,000 [b]	1.532	107.24	0.31
Zinc	70,000 [b]	6.965	487.55	0.69
Selenium (Se)	400 [b]	0.151	10.57	2.64

a) : WHO, 2006
b) : FDA, January 2013

5. Discussion

The safety of dairy products ends when toxic compounds and environmental pollutants (especially heavy metals) concentrations increase. Detection, estimation and comparison of heavy metals levels in dairy products with standard, are needed to establish their safety. (Hegart PV 2008) This issue has been a subject of several studies all over last decades. (WHO 2006) The highest detected values for toxic metals in the current study were those of Pb (7.421 ppm), while the lowest concentrations were of Cd (0.004 ppm).

Al had a highest detected level of (2.74 ppm). As for the essential metals, Se and Zn had highest levels of (0.09 ppm and 31.64 ppm) consecutively, followed by Cu (5.013 ppm). Malhat et al. in 2012 reported lead and cadmium had mean levels of 4.404 and 0.288 ppm correspondingly, in cow milk collected from different sites in El-Qaliubiya governorate, Egypt. These levels are less than values detected in the current study for Pb and Cd simultaneously (7.421, and 5.013 ppm). Fatima et al. in 2005, reported that the Pb was determined in their studied samples, but with concentrations well below the limit defined by WHO in 2006. Based on the notifications from the Agency for Toxic Substances and Disease Registry (ASTDR 2006), regarding the determination of the maximum levels of contaminants in food products, Al content should not exceed l5mg/kg. (ASTDR 2006) According to many authors, Al dietary intake must not exceed 6 mg/day to avoid its potentially toxic effects. (Ysart G et al. 2000, Navaro I and Alvarez JI 2003) Dietary Al intake in USA was estimated by Pennington and Schoen in 1995, to be 0.7-11.5 mg/day in children, 8-9 mg/day in adult males and 7 mg/day in adult females. Ysart et al. in 2000 estimated the mean dietary Al intake in the United Kingdom as 3.4 mg/day. A tolerable daily intake (TDI) for Al of 1 mg/kg of body weight per day has been established by an international committee of experts under the auspices of the WHO in 2006.

Lead content in cheese samples are usually high due to the Pb-binding characteristic of casein. Because of the preferential affinity of metals for the amount of the casein fraction in milk, the concentration of the metals in yoghurt is lower when compared to milk. (Coni E et al. 1996)

Ayar A et al. in 2009 analyzed Pb concentration dairy products and milk consumed in Turkey. According to their study the concentration range of Pb was reported as 0.09 - 0.19 mg/ kg. In another study, conducted by Tajkarimi et al in 2008, lead content was estimated in raw milk collected from different regions of Iran. Accordingly, the mean level of Pb content obtained from 97 samples had a range from 1.0 to 46 ng/mL. These results are by far less than Pb levels in the current study that ranged between 0.018 - 7.421 mg/ kg.

The mean concentrations of Cd in the analyzed milk and dairy products in the current study ranged between 0.004 and 0.672 mg/

kg. This is more than the levels recorded by Rahimi E in 2013, where Cd concentrations in cow milks collected from different regions in Iran ranged between 0.92 to 0.74 ng / mL.

Additionally, Ayar et al. in 2009 reported the concentration of Cd in raw milk, yoghurt and white cheese as 0.002–0.03, 0–0.33 and 0– 0.038 mg/ kg, respectively, which are less than results, recorded in the current study. The main sources of Cd in animal feed in domestic animals are crops, trace element premixes, fish meal and minerals such as, limestone and phosphate. (Bilandzic N et al. 2011) The maximum permissible level of Cd in dairy products has been reported to be 0.02 mg /kg of wet weight in Turkey and 1.0 mg/kg of wet weight in Malaysia. (Ayar A et al. 2009, Food Law of Ministry of Health, Malaysia in 2010).

Trace elements such as Cu, Se and Zn, are known to have vital role in normal growth. But when the amount of intake exceeds the determined levels, they may produce toxic effects. [(32)] Our data demonstrated that the Zn content in raw and pasteurized milk samples were lower than those reported by Licata et al. and Soares et al. in 2010. The content of Cu in raw milk was less than the amount reported by Bilandzic et al. in 2011, and Licata et al. in 2012; but more than those reported by Khan et al. in 2014 The content of Se was less than that given for milk and dairy products in the research conducted by Ayar, et al. (2009) in Turkey Large amounts of Se can have injurious consequences on our health such as hair loss, brittle nails and many other side effects.

The mean Zn concentrations in the current study ranged between (1.541- 22.04). In another study conducted on milk samples collected from great Cairo over on year, its level ranged from 4.1 to 5.45 ppm with an average 4.25 ppm. (Malhat F et al. 2012) Concentrations of Zn were less than the normal levels adopted by the Japanese; 4 mg/ kg, and Chinese; 2.5–6.7 mg /kg. (Qin L et al. 2009) This parameter is important because Zn has many functions in the body and its deficiency leads to disruption in different physiological functions. (Soares VA et al. 2010) Cu contents in the present study were slightly higher when compared with the Japanese and Chinese standards. (Qin L et al. 2009) Apart from the lead which was so close to maximum permissible limit, all products examined, were not even close to permissible levels of other metals analyzed.

6. Conclusions

Toxic adverse health effect due to the consumption of milk and dairy products in Alexandria, Egypt are unlikely in long term exposure consumers. Egypt must have annual risk assessment evaluating programs in food safety. This would bring Egypt into line with international best practices in food safety, increasing the efficiency and effectiveness of food safety programs along the entire Egyptian food chain, including food imports, food exports and the tourist industry. This will also allow food analysis laboratories to build expertise and facilities to analyse foods based on food safety risks and, finally Egyptian consumers can be more confident of the safety of the Egyptian food supply in their market. Additionally, it is suggested that subsequent studies should be conducted on heavy metals contamination in different stages of milk industrial preparation to find out factors that might be involved in heavy metals contamination.

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