The Preliminary Content Of Heavy Metal Residues In Raw Cows Milk And Its Distribution In Some Dairy Products

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ABSTRACT

A total of 108 random samples (36 each of raw cows milk, cream and soft cheese laboratory manufactured from the same milk) were collected from different outlets in Dakahlia Governorate. The collected samples were analyzed for the detection of lead, cadmium, mercury, copper and zinc by using Atomic Absorption Spectrophotometry.

The analyzed data revealed that the mean values in the examined raw milk, cream and cheese samples were (1.970, 0.492 and 1.182) ppm lead , (0.353, 0.088 and 0.2118) ppm cadmium , (0.088, 0.022 and 0.0528) ppb mercury, (0.024, 0.006 and 0.0144) ppm copper and (1.266, 0.316 and 0.7596) ppm zinc .

The statistical analysis indicated that 100% of the examined samples contained lead, cadmium and mercury above the recommended permissible limits.On the other hand, all the examined samples contained copper and zinc below the recommended permissible limits. Moreover, the consumption of 200 ml.cow's milk per day contributed about 78.80, 100.8, 0.352, 0.137 and 0.362%, respectively of the ADI recommended by the FAO/WHO. While the consumption of 100 gm. cream or cheese per day contributed about (9.84, 23.64), (12.57, 30.26), (0.0044, 0.0106),(0.0017, 0.0041)and (0.045, 0.10850)%, respectively of the ADI recommended by the FAO/WHO.

INTRODUCTION

Milk and milk products represent an important part of the human food especially children's diet. So, the contamination of milk and its products by heavy metals is one of the major threats confronting the public health *(1)*.

The animals receive the heavy metals through air , water and feeds whereas the ingestion of contaminated feed stuffs has been considered as the main source of metal residues in the secreted milk *(2 & 3)*. The post-secretion contamination of milk arises from the processing equipment, reagents, accidental contamination during storage and marketing besides leaching from containers *(4)*.

Heavy metals are recognized as cumulative toxic substances due to its low elimination rates from the body. Moreover, the heavy metals could not be metabolized, thus they persist in the body and exert their toxic effects which result in serious health hazards to human , depending on their levels of contamination *(5)*.Among these metals, lead, cadmium and mercury have a great concern due to a variety of their uses that increases their level in environment .They cause several clinical problems due to their competiton with the essential elements for binding sites and their interference with the sulphahydryl groups and structural protein *(6)*. Zinc and copper are essential metals and important in the maintenance of normal physiological function of the body; but in large concentrations , it may cause toxic effects that tend to be more complicated than that of the non essential metals *(7)*.Therefore, this work was undertaken to determine the heavy metal residues in milk and some dairy products collected from Dakahlia Governorate . The control of such pollution was considered .

MATERIALS AND METHODS

1- Collection of samples:

A total of 108 random samples (36 each of raw cows milk, cream and soft cheese laboratory manufactured from the same milk) were collected from different outlets in Dakahlia Governorate along the main road of heavy traffic (El-Mansoura-Zagazig). The raw milk samples were collected in polyethylene bags and taken to the laboratory without delay. Each sample was labeled to identify the source, site and date of sampling. Delayed samples were stored in ice bag.

2- Cream separation: By using modified cream separator.

3- Laboratory manufacture of soft cheese: according to the method recommended by *(8)*.

4-Preparation of samples: A measured volume (10cc) of thoroughly homogenized raw milk and cream samples was transferred in clean and acid washed screw capped digestion tubes. Also, each cheese sample was thoroughly mashed in clean and acid washed mortar and a measured weight (5gm) was transferred into clean and acid washed screw capped digestion tubes. All digestion tubes were identified for examination.

5- Analysis of the prepared samples: Each prepared raw milk, cream or cheese sample was digested according to *(9)* , 10 ml solution of concentrated nitric acid and perchloric acid (1:1) were added to each sample. The samples were cold digested overnight followed by mild increase in temperature till heating at 100°C in water bath for 3-4 hours. 4-5 drops of H2O2 30% were added and heating continued till the brown nitrous gasses were expelled and the specimens became clear. After cooling, each digest was diluted to 25 ml. with deionized water and filtered through Whatmann filter paper No. 42. The clear filtrate of each sample was kept in refrigerator to avoid evaporation.

The determination of mercury in all samples was carried out according to **(10 & 11)**. From the same collected samples another prepared samples were digested with 10 ml solution of concentrated HNO3 and H2SO4 (1:1) in screw capped digestion tubes. Heating on the water bath at 50°C was continued for 15 hours until the evolution of the brown nitrous fumes ceased. After complete digestion, each cold digest was diluted with deionized water to 25 ml and filtered through Whatmann filter paper no. 42. All the filtered samples were analyzed by using "perkia-Elmer Atomic Absorption Spectrophotometer model 2380 equipped with Mercury Hydride system (MHS), USA, 1998" at the microanalytical laboratory, Department of chemistry, Faculty of science, El-Mansoura university, Egypt. For determination of Hg, Flameless Atomic Absorption Spectrophotometer (cold vapour Technique) was used. The analytical detection limits of lead, cadmium, mercury, copper and zinc for the used instrumentation were 0.02 ppm, 0.0006 ppm, 0.01 ppb, 0.003 ppm and 0.002 ppm., respectively.

6- Calculation of the daily metal intake: The daily metal intake was estimated from the consumption of milk, cream and cheese. The mean data of the present study were combined with the consumption data obtained from Nutrition Institute, Cairo, A.R.E., 1996. Comparison of the Acceptable Daily Intake (ADI) values of heavy metals was recommended by *(12)* . It was compared with the calculated daily metal intake from milk, cream and cheese.

RESULTS

Table (1): The heavy metal residues in the examined raw milk samples (N=36).

Metals	Positive samples		Min.	Max.	Mean±S.E.
	No.	%			
Lead (ppm)	36	100.0	1.360	3.720	1.970±0.123
Cadmium (ppm)	36	100.0	0.120	0.560	0.353±0.019
Mercury (ppb)	36	100.0	0.060	0.130	0.088±0.003
Copper (ppm)	36	100.0	0.024	0.024	0.024±0.000
Zinc (ppm)	36	100.0	0.020	3.800	1.266±0.173

*N: The number of the examined samples.

Table (2): Frequency distribution of heavy metals in the examined raw milk samples.

Metals	Permissible limit mg/Kg	Within PL.		Over PL.	
		No. of samples	%	No. samples	%
Lead	0.2a	0	0	36	100.0
Cadmium	0.05a	0	0	36	100.0
Mercury	0.01a	0	0	36	100.0
Copper	0.4b	36	100.0	0	0
Zinc	5.0b	36	100.0	0	0

a : **(2)** b : **(13)**

Table (3): Comparison of the Acceptable Daily Intake (ADI) values of the heavy metals with the calculated daily intake from raw milk.

Metals	ADI(a) ug/70 Kg person	Mean conc. of metals (ug/L) in present study	Calc. daily intake of metals from consumption of 200 ml milk /day (b)	
			ug/day/person	%
Lead	500	1.970	394	78.80
Cadmium	700	0.353	70.6	100.8
Mercury	50	0.088	0.0176	0.352
Copper	35000	0.024	9.8	0.137
Zinc	70000	1.266	253.2	0.362

a: **(12).** b: **(14).**

Table (4): The heavy metal residues in the examined cream and soft cheese samples. (N= 36)

Metals	Cream samples				Cheese samples			
	No. of positive samples	Min.	Max.	Mean ±S.E.	No. of positive samples	Min.	Max.	Mean ±S.E.
Lead (ppm)	36	0.340	0.930	0.492± 0.030	36	0.816	2.232	1.182 ± 0.0738
Cadmium (ppm)	36	0.030	0.140	0.088± 0.004	36	0.072	0.336	0.2118 ± 0.0114
Mercury (ppb)	36	0.016	0.033	0.022± 0.001	36	0.036	0.078	0.0528 ± 0.0018
Copper (ppm)	36	0.006	0.006	0.006± 0.000	36	0.0144	0.0144	0.0144 ± 0.000
Zinc (ppm)	36	0.050	0.950	0.316± 0.043	36	0.012	2.28	0.7596 ± 0.1038

Table (5): Comparison of the Acceptable Daily Intake (ADI) values of heavy metals with the calculated daily intake from cream.

Metals	ADI(a) ug/70 Kg person	Mean conc. of metals (ug/kg) in present study	Calc. daily intake of metals from consumption of 100 gm cream /day (b)	
			ug/day/person	%
Lead	500	0.492	49.2	9.84
Cadmium	70	0.088	8.8	12.57
Mercury	50	0.022	0.0022	0.0044
Copper	35000	0.006	0.6	0.0017
Zinc	70000	0.316	31.6	0.045

Table (6): Comparison of the Acceptable Daily Intake (ADI) values of heavy metals with the calculated daily intake from soft cheese.

Metals	ADI(a) ug/70 Kg person	Mean conc. of metals (ug/kg) in present study	Calc. daily intake of metals from consumption of 100 gm cheese /day (b)	
			ug/day/person	%
Lead	500	1.182	118.2	23.64
Cadmium	70	0.2118	21.18	30.26
Mercury	50	0.0528	0.0053	0.0106
Copper	35000	0.0144	1.44	0.0041
Zinc	70000	0.7596	75.96	0.1085

Table (7): Frequency distribution of heavy metals in the examined soft cheese samples.

Metals	Permissible limit mg/Kg	Within PL.		Over PL.	
		No. of samples	%	No. of samples	%
Lead	0.3	0	0	36	100.0
Cadmium	0.05	0	0	36	100.0
Mercury	0.02	0	0	36	100.0
Copper	0.3	36	100.0	0	0
Zinc	20	36	100.0	0	0

DISCUSSION

Heavy metals in the examined raw milk samples:

1- lead:

The Pb concentrations , in the present work ranged from 1.360 to 3.720 ppm with a mean of 1.970 ppm. Nearly similar findings were reported by *(15)*. Higher concentrations were recorded by *(16 & 17)* , while lower levels were obtained by *(18 & 19)*. Thirty-six (100%) milk samples contained Pb above the recommended PL. (0.2ppm) as mentioned by *(2)*. The Higher Pb concentrations in the raw milk may be attributed to the excessive exposure of the lactating cows to environmental lead discharged from heavy traffics, contamination of the feeding stuffs and water and mobilization of lead from its stores in the skeleton of lactating animals. The average Pb concentration in the raw milk samples was 1.970 ug/L, which gave daily

intake of about 394ug. for adult person from consumption of 200 ml. milk per day and this contributed about 78.80% of the ADI recommended by *(12)*.

2-Cadmium:

The mean concentration of Cd was 0.353 ppm in the examined raw milk samples with a maximum concentration of 0.560 ppm and a minimum concentration of 0.120 ppm. Higher levels were detected by *(17 & 19)*, while lower concentrations were reported by *(20 & 21)*. All the examined samples revealed Cd concentrations above the recommended PL .(0.05 ppm) cited in *(2)*. The presence of Cd at a high concentration in cow's milk may be due to the consumption of contaminated feed stuffs and water, specially cows reared in polluted regions *(22)*. The average concentration of Cd in the examined raw milk was 0.353 ug/L, which gave daily intake of about 70.6ug for the adult person from consumption of 200 ml milk per day and this amount contributed about 100.8% of ADI recommended by *(12)*.

3-Mercury:

The Hg concentration in the examined raw milk samples ranged from 0.060 to 0.130 ppb with a mean level of 0.088 ppb. *(23)* recorded Hg concentration in high level. Meanwhile, lower concentration was recorded by *(24)*. All the examined raw milk samples (100%) showed Hg concentration above the recommended PL. (0.01 ppm) recorded by *(2)*. The presence of Hg in cow's milk may be attributed to the consumption of polluted feed stuffs and water as well as the excessive use of fungicides and pesticides that contain this metal. The mean value of Hg in the examined milk samples was 0.088 ug/L, which gave daily intake of about 0.0176 ug/adult person from the consumption of 200 ml. milk per day and this representing about 0.352% of ADI recommended by *(12)*.

4- Copper:

The mean concentration of copper was 0.024 ppm. Higher copper concentration was recorded by *(18 , 17 & 19)*. All the examined samples (100%) showed copper concentration below the PL.(0.4ppm) according to *(13)*. The presence of a low level of copper in cow's milk may be attributed to the gradual decline of copper content of milk from the first day of lactation to the normal level through two months *(25)*. The calculated daily intake of copper was 9.8 ug/adult person from the consumption of 200ml. milk per day and this amount represents 0.137 % of ADI recommended by *(12)*.

5-Zinc:

The Zn concentration in the examined raw milk samples ranged from 0.020 to 3.800ppm with a mean of 1.266 ppm. These results agreed with those obtained by Nasef (2000). Higher levels were recorded by *(20& 24)*, while lower values were reported by *(26)*. All the examined raw milk samples showed Zn values below the recommended PL. (5.0ppm) established by *(13)*. The low level of Zn may be attributed to the fact that Zn is a normal component of milk and the data on Zn levels in milk showed a wide variation with an average of 4.4ppm *(27)*. The average concentration of Zn in the raw milk samples was 1.266 ug/L,which gave daily intake of about 253.2 ug for the adult person from the consumption of 200 ml milk per day and this represented about 0.362% of the ADI recommended by *(12)*.

Heavy metals in the examined cream and soft cheese:

1- lead:

The Pb concentrations in the examined cream and soft cheese samples were 0.492 and 1.182 ppm, respectively. Higher values were reported by *(28 & 29)*, while lower levels were reported by *(30)*. The average concentrations of lead in the cream and soft cheese samples were 0.492 and 1.182 ug/Kg, respectively, which gave daily intake of about 49.2 and 118.2 ug for the adult person from consumption of 100 gm per day and this represented about 9.84 and 23.64%, respectively of the ADI as recommended by *(12)*. All the examined soft cheese samples (100%) contained Pb concentrations above the recommended PL. (0.3ppm) as established by *(31)*.

2- Cadmium:

The average levels of Cd in the examined cream and soft cheese samples were 0.088 and 0.2118 ppm, respectively. Higher levels were reported by *(32 & 29)*. While lower values were reported by *(33 & 30)*. The

average levels of Cd in cream and soft cheese samples were 0.088 and 0.2118 ug/Kg, which gave daily intake of about 8.8 and 21.18 ug for the adult person from consumption of 100 gm. per day and this contributed about 12.57 and 30.26%, respectively of the ADI recommended by *(12)*. All the examined cheese samples contained Cd level above the recommended PL. (0.05 ppm) as established by *(31)*.

3- Mercury:

The average concentrations of Hg in the examined cream and soft cheese samples were 0.022 and 0.0528 ppb, respectively. Nearly similar data were reported by *(29)*. Meanwhile higher values were recorded by *(23 & 34)*. The average levels of Hg in cream and soft cheese samples were 0.022 and 0.0528 ug/Kg, which gave daily intake of about 0.0022 and 0.0053 ug for the adult person from consumption of 100 gm per day and this contributed about 0.0044 and 0.0106%, respectively of the ADI. All the examined cheese samples contained Hg values above the recommended PL. (0.002 ppm).

4- Copper:

The copper concentration in the examined cream and soft cheese samples were 0.006 and 0.0144 ppm, respectively. Relatively higher values were reported by *(30 & 35)*. The average values of copper in cream and soft cheese samples were 0.006 and 0.0144 ug/Kg, which gave daily intake of about 0.6 and 1.44 ug for the adult person from the consumption of 100 gm per day and this contributed about 0.0017 and 0.0041%, respectively of ADI. All the examined cheese samples contained copper levels below the recommended PL. (0.3 ppm).

5- Zinc:

The mean concentrations of Zn in the examined cream and soft cheese samples were 0.316 and 0.7596 ppm., respectively. Higher values were reported by *(30 & 29)*. The average concentrations of zinc in the examined cream and soft cheese samples were 0.316 and 0.7596 ug/kg which gave daily intake of about 31.6 and 75.96 ug for the adult person from consumption of 100 gm per day and contributed 0.045 and 0.1085% respectively of ADI.All the examined cheese samples contained Zn levels below the recommended PL. (20ppm).

Public health importance:

lead:

It has a large affinity for the thiol and phosphate containing ligands, inhibiting the biosynthesis of heme and thereby affects the membrane permeability of the kidneys, liver and brain cells which reduces the function or completely breakdown these tissues *(39)*. Therefore, the CNS, kidneys, liver and hematopoietic system are important targets of lead toxicosis .

Cadmium:

It acts on the sulfhydryl groups of the essential enzymes, it binds the phospholipids and interferes with the oxidative phosphorylation as well as it can replace Zn in metalloenzymes which changes its activity *(1)*. Cadmium accumulates mainly in the liver and kidneys *(40)*. The chronic Cd toxicosis included kidney damage with proteinuria, impaired regulation of calcium and phosphates, manifesting bone demineralization, osteomalacia and pathological fractures *(41)*. Moreover, Cd is a possible cause of hypertension, insomnia and testicular atrophy *(42)*.

Mercury:

It is a particular cumulative poison and acts as inhibitor for numerous enzymes. Moreover, it causes sever kidney damage in both man and animals *(43)*.

Copper:

It is an essential element for several enzymes. The gastrointestinal absorption of Cu is normally regulated by body store and most of the Cu is stored in the liver and bone marrow where it is bound to metallothioneine *(44)*. The maximum daily intake of Cu is 0.5mg/kg. body weight *(12)* so, the increased of Cu consumption above the recommended permissible limit may become a threat to man *(45)*.

Zinc:

It is an essential constituent or cofactor for more than 200 mettalloenzymes, hormones and hormone receptors, protein, neuropeptides and polynucleotides *(46)*. Excessive exposure

to Zn is relatively uncommon and requires heavy exposure. Zn does not accumulate with continued exposure but the body content is modulated by homeostatic mechanisms that act principally on the absorption and liver level *(47)*.

It could be concluded that the heavy metal residues were found in the raw cows milk , cream and soft cheese beyond the permissible limits for human consumption , which may constitute a possible public health hazard. Therefore , the monitoring of the heavy metals in milk and its products should be done to protect the consumer from exposure to unacceptable levels of these metals.

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الملخص العربي

المحتوى الأولى لبقايا المعادن الثقيلة في لبن الأبقار الخام وتوزيعها في بعض منتجات الألبان

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أجري هذا البحث لتقدير بقايا المعادن الثقيلة في اللبن البقري الخام و القشدة و الجبن الطري المصنع معمليا من هذا اللبن و المنتج من المزارع المختلفة بمحافظة الدقهلية ومقارنة تركيز هذه المعادن الثقيلة بالحدود المسموح بها. تم تجميع عدد ١٠٨ عينة عشوائية (٣٦ عينة من كل من اللبن الخام و القشدة و الجبن الطري المصنع من هذا اللبن) و بعد ذلك تم تجهيزها و هضمها ثم قياسها باستخدام جهاز الامتصاص الذري الطيفي.وقد أسفرت الدراسة عن تواجد الرصاص و الكادميوم و الزئبق و النحاس و الزنك في عينات اللبن البقري و القشدة و الجبن الطري بمتوسط قدره (١,٩٧٠، ٠,٤٩٢، ١,١٨٢) جزء في المليون &(٠,٣٥٣، ٠,٠٨٨، ٠,٢١١٨) جزء في المليون& (٠,٠٨٨، ٠,٠٢٢، ٠,٠٥٢٨) جزء في البليون & (٠,٠٢٤، ٠,٠٠٦، ٠,٠١٤٤) جزء في المليون و (١,٢٦٦، ٠,٣١٦، ٠,٧٥٩٦) جزء في المليون علي التوالي.وقد اتضح من التحليل الإحصائي للعينات أن معدلات تركيز الرصاص و الكادميوم و الزئبق قد تجاوزت الحدود المسموح بها في ١٠٠% من إجمالي العينات بينما لم يتجاوز النحاس و الزنك الحدود المسموح بها في كل العينات وقد اتضح أيضا أن المأخوذ اليومي من الرصاص و الكادميوم والزئبق و النحاس و الزنك للإنسان البالغ من اللبن تمثل ٧٨,٨، ١٠٠,٨ ، ٠,٣٥٢، ٠,١٣٧ و ٠,٣٦٢ % من المأخوذ اليومي المسموح به عالميا أما المأخوذ اليومي من نفس العناصر من القشدة و الجبن الطري المصنع حيث كان يمثل (٩,٨٤، ٢٣,٦٤) & (١٢,٥٧، ٣٠,٢٦) & (٠,٠٠٤٤، ٠,٠١٠٦)&(٠,٠٠١٧، ٠,٠٠٤١) و (٠,٠٤٥، ٠,١٠٨٥) % علي التوالي من المأخوذ اليومي المسموح به عالميا. هذا ولقد تم منا قشة النتائج وخطورة تواجد بقايا المعادن الثقيلة في الألبان ومنتجاتها على الصحة العامة.