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Heavy Metals Residues in Some Dairy Products

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Abstract

A total of Sixty random samples of dairy products (20 each of condensed milk, evaporated milk and infant formula) were collected from different outlets and pharmacies in Alexandria governorate, Egypt. All examined samples were analyzed by Atomic Absorption Spectrophotometer to determine heavy metals residues and trace elements [lead (Pb), Cadmium (Cd), Copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn)]. The results obtained revealed that extremely toxic heavy metals (Pb and Cd) were detected above the recommended daily intake in infant formula, while were acceptable when compared with average daily intake in examined condensed and evaporated milk samples. Public health significance of heavy metal was discussed.

1. INTRODUCTION:

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 children and adults around the World (Enb *et al*., 2009).

Milk and milk products contain more than twenty different trace elements most of them are essential and very important such as copper, zinc, manganese and iron (Pennington *et al*., 1995).These metals are co-factors in many enzymes and play an important role in many physiological function and lack of these metals cause disturbances and pathological conditions (Schuhmacher *et al*., 1991).

Although these metals are essential for health, excessive exposure may be hazardous (Akhter, 2004) . High manganese exposure in childhood can result in a neurotoxic syndrome affecting dopamine balance and behavior control (Ukhun *et al* .,1990), toxic level of copper may lead to Wilson's disease (excessive accumulation of Cu in brain and cornea) and Menke's disease (peculiar hair , severe mental retardation , neurological impairment and death before 3 years of age) (Goyer, 1996)

In addition to the essential minerals, milk can contain potentially toxic metals, notably Cd and Pb (Abdulkahaliq *et al*., 2012).

Heavy metals are described as those metals which, in their standard state, have a specific gravity (density) of more than about 5 g/cm^3(Holleman and Wiberg, 2001).

Heavy metals in dairy products may be due to contamination of the original cow's milk, which may be due to exposure of lactating cow to environmental pollution or consumption of feeding stuffs and water (Carl, 1991 and Okada *et al*., 1997). Moreover, raw milk may be exposed to contamination during its manufacture (El-Batanouni and Abo El-Ata, 1996).

The presence of toxic heavy metals as cadmium and lead even in low concentrations, leads to metabolic disorders and serious health problems such as weakness, heart failure, cancer and also affects the kidneys (Licata *et al*., 2004).

Canned dairy products are considered a source of heavy metal toxicity due to migration of metals from can to product it contain during long storage period or damaged can (Oskarsson *et al*., 1995).
Elemental analysis of milk and subsequently dairy product is important for the assessment of the quality of milk as well as to determine any possible contamination through adulteration or by environment (Okada *et al*., 1997).

So, this work was undertaken to determine the toxic and essential heavy metals in examined samples of condensed and evaporated milk as well as the infant formula that collected from Alexandria governorate, Egypt.

2. MATERIAL AND METHOD

1- Collection of samples:

Sixty random dairy product samples {20 of each condensed milk, evaporated milk and infant formula}, were randomly collected from different outlets and pharmacies in Alexandria governorate, Egypt in their original package. All samples were analyzed before their expiry date. Collected samples were taken to the laboratory. Each sample was labeled to identify the source, site and date of sampling.

2- Samples and standards preparation (AOAC, 1995) **:**

Commercial standard solutions for atomic absorption spectrophotometer (AAS), BDH chemicals Ltd., Poole, UK, were used for all metal standard solutions. All reagents were of analytical reagent grade.

3- Analytical parameters:

SHIMADZU Atomic Absorption Spectrophotometer model (AA6650) was used in this work for detection of lead, cadmium , copper , zinc , iron and manganese. The apparatus was adjusted at wave lengths of 217.0 nm for lead, 228.8 nm for cadmium, 324.8 nm for copper, 213.9 nm for zinc, 248.3 nm for iron and 279.5 nm for manganese

4- Statistical analysis:

Values expressed as mean ± SEM of triplicate measurement. Analysis of variance was performed to compare the differences among the groups using (**SAS, 2004) .**

3. RESULTS AND DISSCUSSION

Table (1): Lead concentration (ppm) in examined dairy product samples and comparison with standard (N = 20 of each).

product	Positive Samples				Mean ± SEM	Permissible limit mg/kg (ppm)	Within permissible limit		Over permissible limit	
	No.	%	Min.	Max.			No.	%	No.	%
Condensed milk	14	70	0.34	1.45	0.88 ± 0.08 B	0.02*	6	30	14	70
Evaporated milk	20	100	0.34	2.39	1.17 ± 0.13 A	0.02*	0	0	20	100
Infant formula	18	90	0.17	1.71	0.85 ± 0.10 B	0.02*	2	10	18	90

Means within the same column of different letters are significantly different at($P<0.01$). *Egyptian standards, No7136 / 2010 .

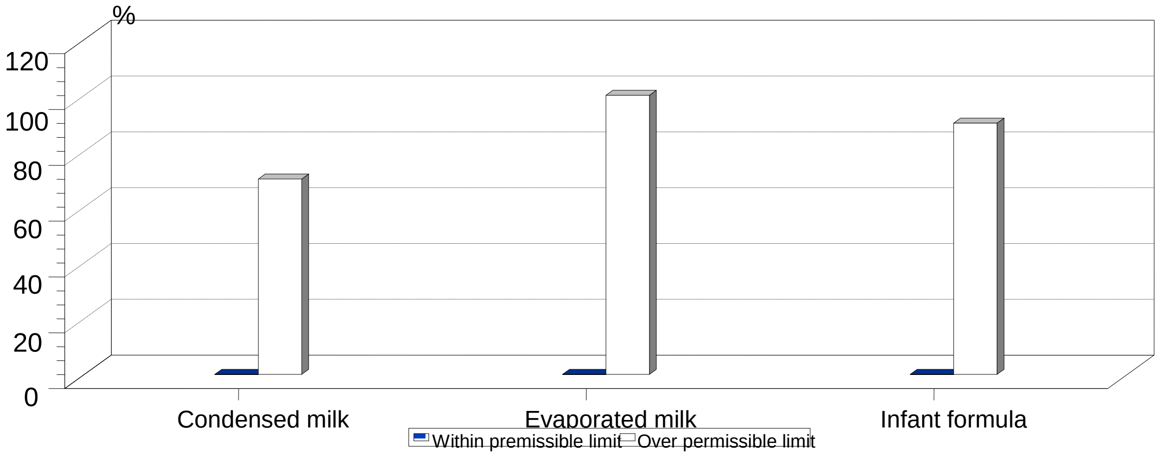


Fig (1): Frequency distribution of lead residues in examined milk products (N=20).

Table (2): Cadmium concentration (ppm) in examined dairy product samples and comparison with standard (N = 20 of each) .

product	Positive Samples				Mean ± SEM	Permissible limit mg/kg (ppm)	Within permissible limit		Over permissible limit	
	No.	%	min	max			No.	%	No.	%
Condensed milk	20	100	0.01	0.10	0.06 ± 0.01 B	0.02*	2	10	18	90
Evaporated milk	20	100	0.02	0.11	0.07 ± 0.01 AB	0.02*	1	5	19	95
Infant formula	18	90	0.01	1.23	0.11 ± 0.06 B	0.02*	5	25	15	75

Means within the same column of different letters are significantly different at (P < 0.01).

*** CAC, 2004.**

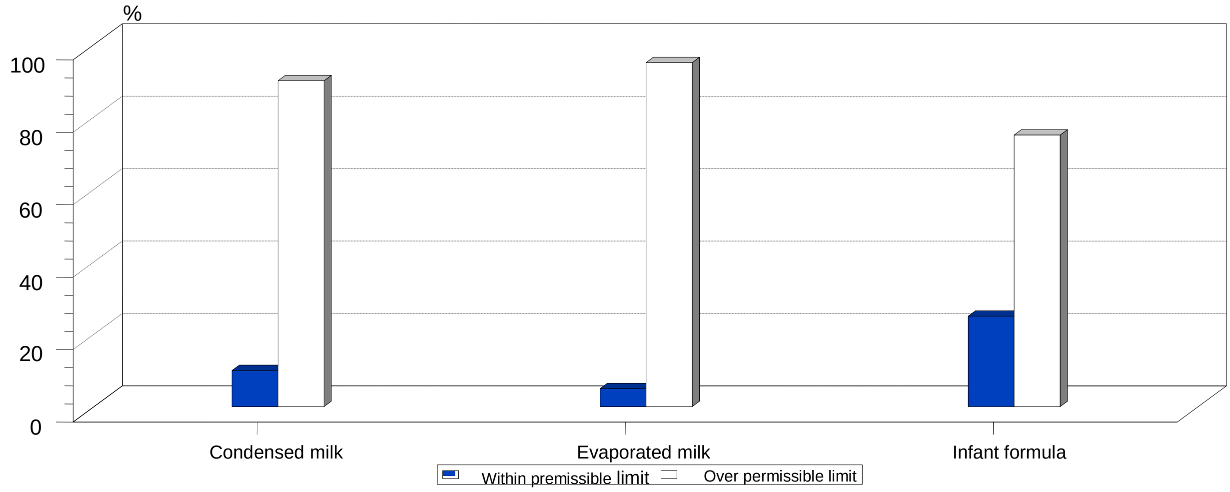


Fig (2): Frequency distribution of Cadmium residues in examined milk products (N=20).

Table (3): Copper concentration (ppm) in examined dairy product samples and comparison with standard (N=20 of each) .

product	Positive Samples				Mean ± SEM	Permissible limit mg/kg (ppm)	Within permissible limit		Over permissible limit	
	No.	%	min	Max			No.	%	No.	%
Condensed milk	20	100	2.67	28.42	5.19 ± 1.24 A	----	---	---	---	---
Evaporated milk	20	100	0.96	20.89	4.52 ± 1.22 B	0.2*	0	0	20	100
Infant formula	20	100	0.89	8.45	4.51 ± 0.34 B	0.5**	0	0	20	100

Means within the same column of different letters are significantly different at (P < 0.01).

*** Holland et al ., 1995. ** commission Regulation (EU), No 600/2010.**

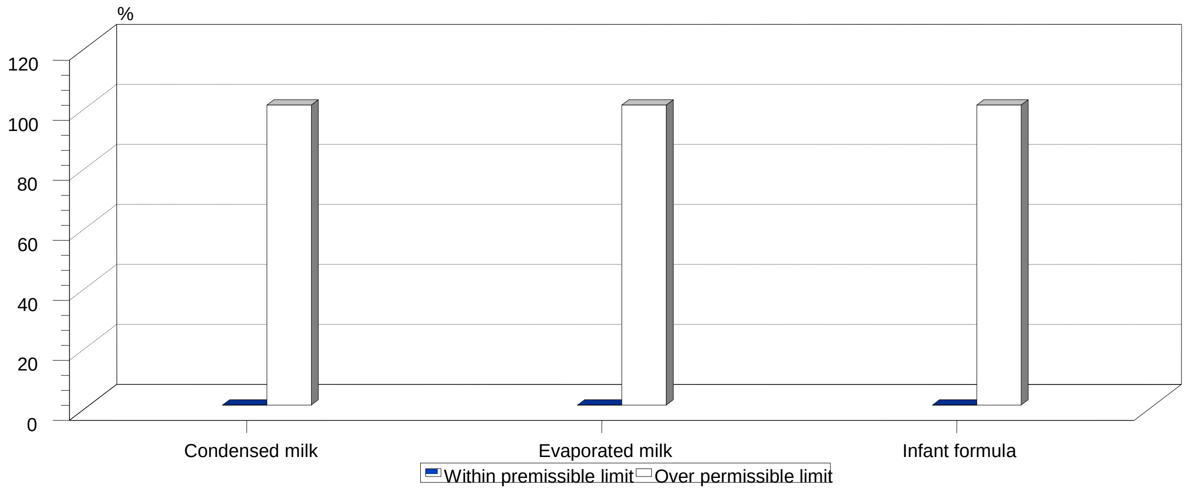


Fig (3): Frequency distribution of Copper residues in examined milk products (N=20).

Table (4): Zinc concentration (ppm) in examined dairy product samples and comparison with standards (N=20 of each).

product	Positive Samples				mean± SEM	Permissible limit mg/kg (ppm).	Within permissible limit		Over permissible limit	
	No.	%	Min.	Max.			No.	%	No.	%
Condensed milk	18	90	0.62	16.56	9.92 ± 1.26 B	10*	9	45	11	55
Evaporated milk	19	95	0.27	18.77	5.56 ± 1.42 C	9*	15	75	5	25
Infant formula	20	100	1.68	17.75	13.42 ± 0.79 A	5**	1	5	19	95

Means within the same column of different letters are significantly different at (P < 0.01).

*** Holland et al ., 1995 .** ****commission Regulation (EU), No 600/2010 .**

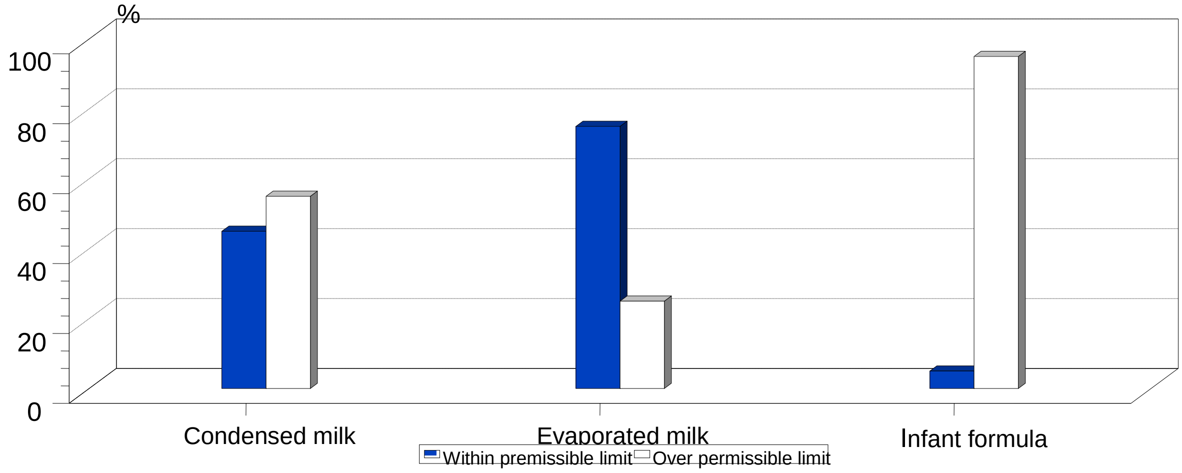


Fig (4): Frequency distribution of Zinc residues in examined milk products (N=20).

Table (5): Iron concentration (ppm) in examined dairy product samples and comparison with standards (N=20 of each).

product	Positive Samples				Mean ± SEM	Permissible limit mg/kg (ppm)	Within permissible limit		Over permissible limit	
	No.	%	Min.	Max.			No.	%	No.	%
Condensed milk	20	100	2.78	20.74	8.61 ± 1.14 B	2.3*	0	0	20	100
Evaporated milk	20	100	3.63	42.84	9.77 ± 1.94 B	2.6*	0	0	20	100
Infant formula	20	100	5.00	35.24	24.88 ± 1.77 A	72**	20	100	0	0

Means within the same column of different letters are significantly different at (P < 0.01).

*** Holland et al ., 1995.** **** European Commission1996.**

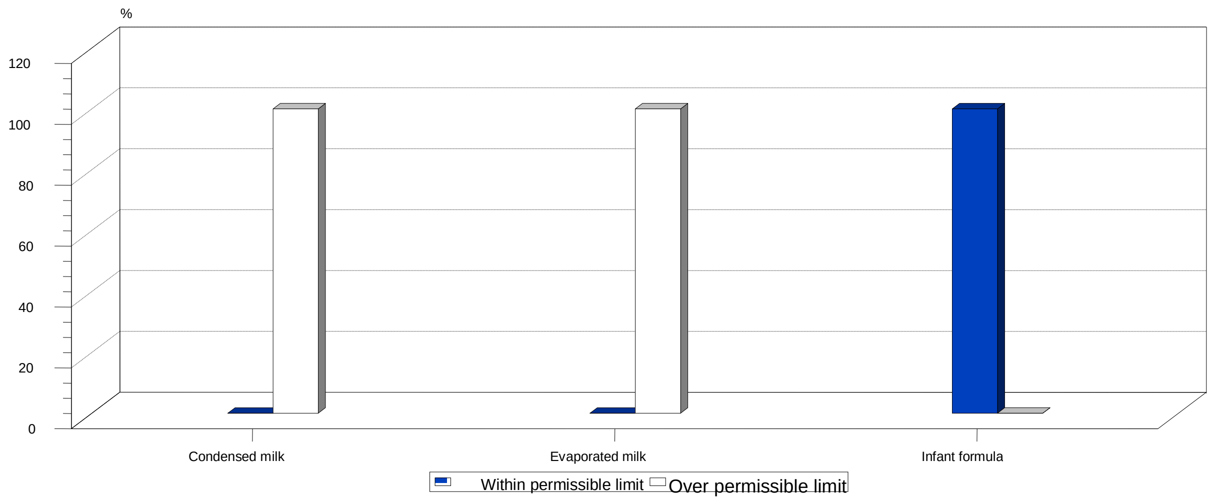


Fig (5): Frequency distribution of Iron residues in examined milk products (N=20).

Table (6): Manganese concentration (ppm) in examined dairy product samples and comparison with standard (N=20 of each) .

product	Positive Samples				Mean ± SEM	Permissible limit mg/kg (ppm)	Within permissible limit		Over permissible limit	
	No.	%	Min.	Max.			No.	%	No.	%
Condensed milk	20	100	0.02	0.96	0.51 ± 0.07 B	---	---	---	---	---
Evaporated milk	20	100	0.03	1.50	0.47 ± 0.09 B	---	---	---	---	---
Infant formula	20	100	0.13	2.03	0.76 ± 0.08 A	0.65*	8	40	12	60

Means within the same column of different letters are significantly different at ($P < 0.01$).

***European Commission Directive 2006/141/EC .**

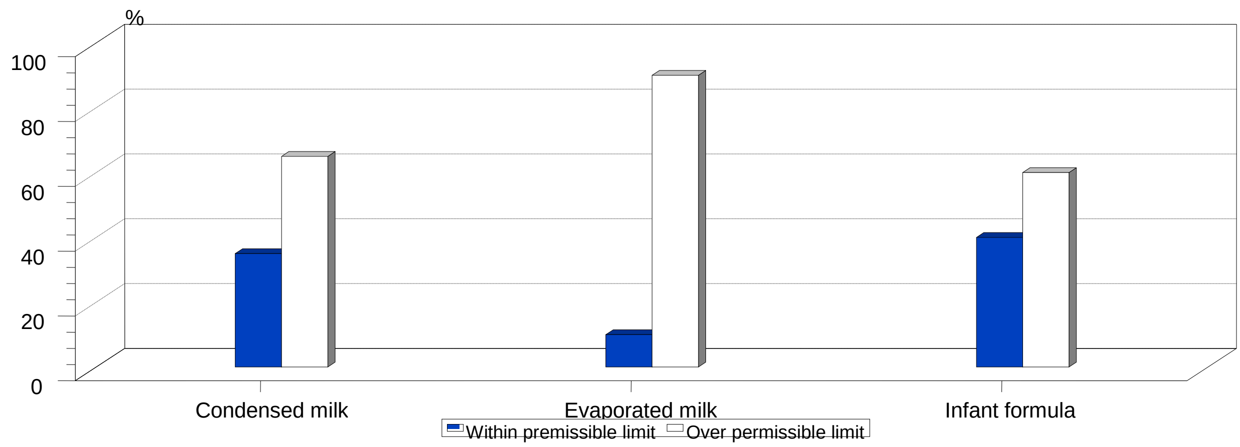


Fig (6): Frequency distribution of Manganese residues in examined milk products (N=20).

Table (7): Comparison of Acceptable Daily Intake (ADI) value of heavy metals and trace elements with the calculated daily intake from condensed milk.

Metals	ADI µg /70 kg person	Mean concentration of metals (µg/kg) in examined samples	Calculated daily intake of metals from consumption of 100 gm condensed milk per day*****	
			µg /day / person	%
Lead	250*	880	88	35.20
Cadmium	70**	60	6	8.6
Copper	3000***	5190	519	17.3
Zinc	15000***	9920	992	6.61
Iron	18000***	8610	861	4.78
Manganese	5000 ****	510	51	1.02

*** Codex Stand 193-1995 .** **** WHO, 1996 .**
***** IMNAS 2000, IMNAS, FNB 2001 .** ****** Perveen et al 2005 .**
******* Nutrition institute 1996 .**

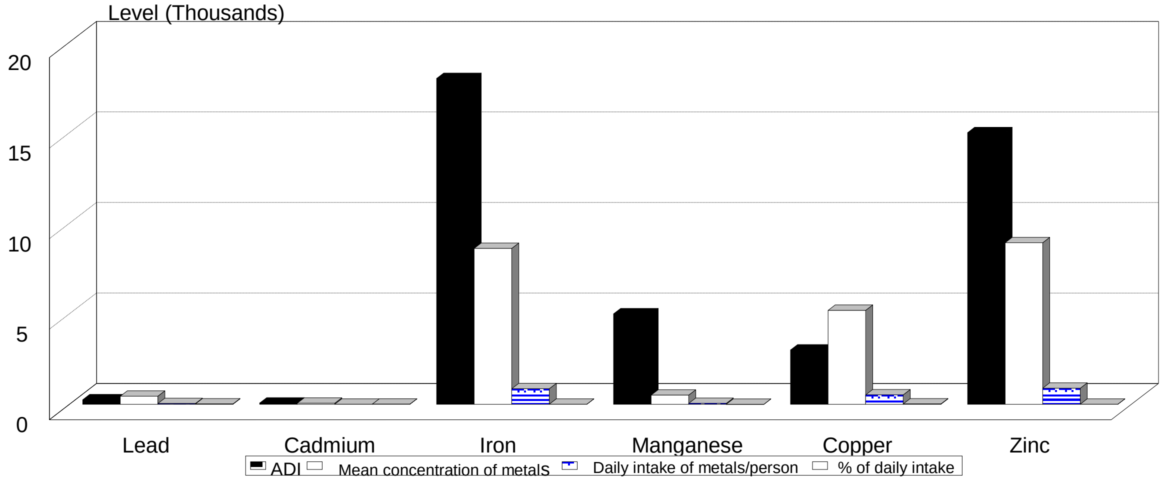


Fig. (7): Comparison of Acceptable Daily Intake (ADI) value of heavy metals and trace elements with the calculated daily intake from condensed milk.

Table (8): Comparison of Acceptable Daily Intake (ADI) value of heavy metals and trace elements with the calculated daily intake from evaporated milk.

Metals	ADI µg /70 kg person	Mean concentration of metals (µg/kg) in examined samples	Calculated daily intake of metals from consumption of 100 ml evaporated milk per day*****	
			µg /day / person	%
Lead	250*	1170	117	46.8
Cadmium	70**	70	7	10
Copper	3000***	4520	452	15.06
Zinc	15000***	5560	556	3.70
Iron	18000***	9770	977	5.43
Manganese	5000****	470	47	0.94

***Codex Stand 193-1995 .** ****WHO, 1996 .**
*****IMNAS 2000, IMNAS, FNB 2001 .** ******Perveen et al 2005 .**
******* Nutrition institute 1996 .**

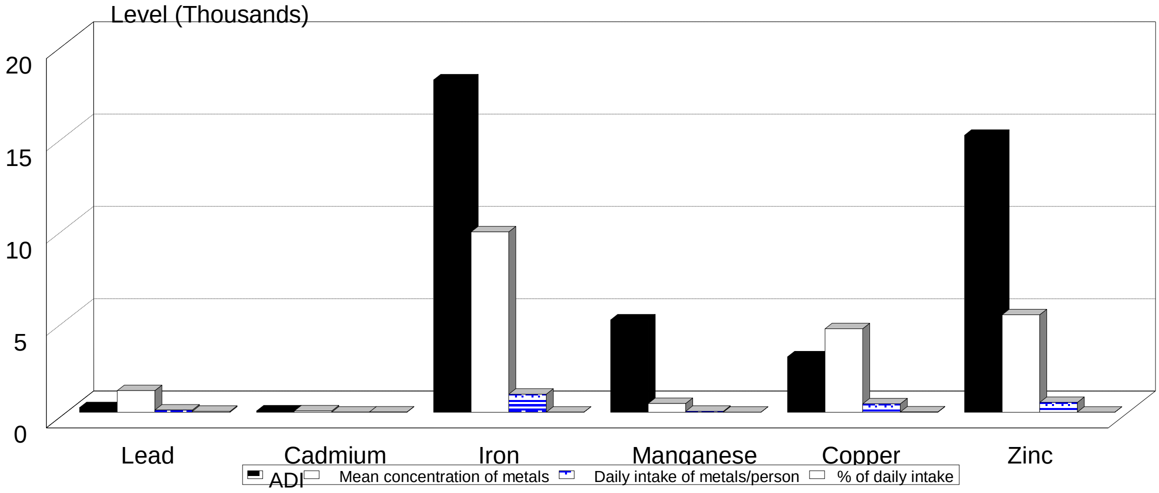
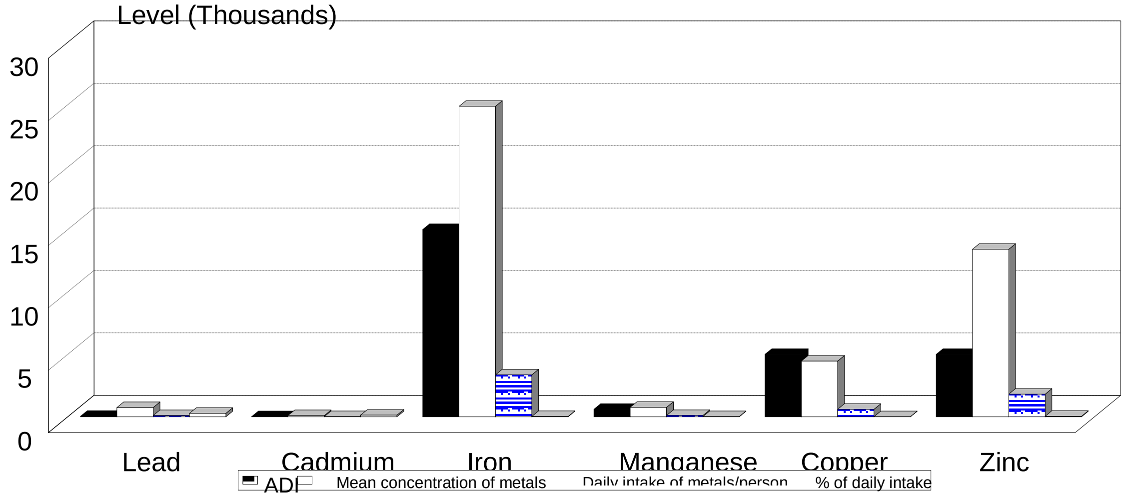


Fig. (8): Comparison of Acceptable Daily Intake (ADI) value of heavy metals and trace elements with the calculated daily intake from evaporated milk..

Table (9): Comparison of Acceptable Daily Intake (ADI) value of heavy metals and trace elements with the calculated daily intake from infant formula .

Metals	ADI µg/10kg 1nfant	Mean concentration of metals (µg/kg) in examined samples	Calculated daily intake of metals from consumption of 135 gm of infant formula per day******	
			µg /day / person	%
Lead	**36***	**850**	**114.75**	**318.75**
Cadmium	**10****	**100**	**13.5**	**135**
Copper	**5000*****	**4460**	**602.1**	**12.04**
Zinc	**5000******	**13420**	**1811.7**	**36.23**
Iron	**15000******	**24880**	**3358.8**	**22.39**
Manganese	**600*******	**760**	**102.6**	**17.1**

***WHO 2000 .** ****WHO 2001 .**
*****WHO 1982 .** ******National Academy of Science, Washington, DC, U.S.A 2004 .**
*******EFSA, 2009 .** ********Feeding tables and dosage recommended by manufactures .**



Analysis of examined dairy products samples indicated their contamination by some heavy metals residues, which considered hazardous impacts on human health. Beyond certain limits, all metals turned to be toxic to human body. This could be applied to essential minerals like Cu ; Zn ; Fe and Mn , as well as non-essential metals and metalloids like Pb and Cd compounds.

Results obtained in Table (1) revealed that, the Pb concentration , in condensed milk ranged from 0.34 to 1.45 ppm with a mean value of 0.88 ± 0.08 ppm . higher concentration were reported by (Salah, 2012) while lower level were reported by (Elena, 1999) , in evaporated milk the Pb concentration ranged from 0.34 to 2.39 ppm with a mean value of 1.17 ± 0.13 ppm . lower level were reported by (Khalil and Seliem, 2013) , while in infant formula the Pb concentration ranged from 0.17 to 1.71 ppm with a mean value of 0.85 ± 0.10 ppm . this result are in agreement with (Salah, 2012), higher result was reported by (Mohamed, 2013) while lower level were reported by (Mohamed and Aigin, 2014) and (Sadeghi, 2014).

From the other hand Table (1) & Fig (1) revealed that 14 (70%) , 20 (100%) and 18 (90%) of condensed milk, evaporated milk and infant formula samples, were contaminated with Pb above the recommended permissible limit (0.02ppm) as established by (Egyptian standards, 2010).

lead piping and lead-lined tanks of water supplies are the sources of lead contamination. lead , due to its wide use in industrial processes , ranks as the metal of largest diffusion through the atmosphere ; one could speculate that this could in part explain the relatively high concentration found . (Carl, 1991 and Okada *et al*., 1997).

The high lead level in examined dairy products samples may be attributed to the fact that the migration of lead from the surface of the can to the products (Ramonaityte, 2001) or may be due to excessive exposure of raw milk to environmental lead pollution (heavy traffic , contamination of feeding stuff, mobilization of lead from it is stores in skeleton of lactating animal .

The obtained data showed in Table (2) illustrated that, the Cd concentration , in condensed milk ranged from 0.01 to 0.10 ppm with a mean value of 0.06 ± 0.01 ppm . higher concentration were reported by (Salah, 2012) while lower level were reported by (Elena, 1999) ,in evaporated milk the Cd concentration ranged from 0.02 to 0.11 ppm with a mean value of 0.07 ± 0.01 ppm . lower level were reported by (Khalil and Seliem, 2013) . In infant formula the Cd concentration ranged from 0.01 to 1.23 ppm with a mean value of 0.11 ± 0.06 ppm . higher concentration were reported by (Mohamed, 2013) while lower level was reported by (Mohamed and Aigin 2014) and (Sadeghi, 2014).

Results gained in Table (2) & Fig (2) revealed that 18 (90%) , 19 (95%) and 15 (75%) of condensed milk, evaporated milk and infant formula samples, respectively, contained Cd above the recommended permissible limit (0.02 ppm) as established by (Codex Alimentarius Commission, 2004).

Milk and dairy products usually contain very low concentration of Cd except when dairy animals consumed contaminated feeds and water. Moreover, contamination during storage, marketing and leaching from containers may be considered as a source of Cd in milk and other dairy products. The distribution change of Cd after heat treatment of milk due to the formation of complexes between the whey proteins and the metal or to the desegregation of the Cd bound to casein micelles. (Cabrera *et al*., 1995 and Okada *et al*., 1997)

Results in Table (3) illustrated that, the Cu concentration , in condensed milk ranged from 2.67 to 28.42 ppm with a mean value of 5.19 ± 1.24 ppm . lower level were reported by (Aytham, 2012) , in evaporated milk the Cu concentration ranged from 0.96 to 20.89 ppm with a mean value of 4.52 ± 1.22 ppm . lower level were reported by (Khalil and Seliem 2013) , while in infant formula the Cu concentration ranged from 0.89 to 8.45 ppm with a mean value of 4.51 ± 0.34 ppm . this result are in agreement with (Sadeghi, 2014), while lower level were reported by (Jolanta, 1996 and Aytham, 2012) .

Results in Table (3) & Fig (3) revealed that 20 (100%) of evaporated milk samples contained Cu above the recommended permissible limit (0.2 ppm) as established by (Holland *et al* ., 1995). Also 20 (100%) of infant formula samples contained Cu above the recommended permissible limit (0.5 ppm) as established by (commission Regulation, 2010) .

The high copper level in examined infant formula samples may be due to the nature of tinned can manufacture (which consists of in 98.8 %, small amount of lead beside soldering of seams with lead-containing paste, copper, cadmium, aluminum from the Al-foil cover and some other metals) capable of participating in electrochemical transformation (Arvanitoyannis and Bosnea, 2004).

Data obtained in (Table 4) illustrated that, the Zn concentration , in condensed milk was ranged from 0.62 to 16.56 ppm with a mean value of 9.92 ± 1.26 ppm . lower level were reported by (Elena, 1999) , in evaporated milk the Zn concentration ranged from 0.27 to 18.77 ppm with a mean value of 5.56 ± 1.42 ppm . lower level were reported by (Khalil and Seliem 2013) , while in infant formula Zn concentration ranged from 1.68 to 17.75 ppm with a mean value of 13.42 ± 0.79 ppm . higher level was reported by (Humaria *et al*., 2015) while lower level reported by (Iwegbuue, 2010).

Table (4) & Fig (4) was revealed that 11 (55%) and 5 (25%) of condensed and evaporated milk samples contained Zn above the recommended permissible limit (10 and 9 ppm , respectively) as established by (Holland *et al* ., 1995). 19 (95%) of infant formula samples contained Zn above the recommended permissible limit (5 ppm) as established by (commission Regulation, 2010) .

Soluble Zinc salts which causing poisoning in animals usually originated from galvanized iron used as piping or drinking utensils . Zinc based paints, with 50 - 55 % Zinc content were common sources of poisoning when cattle take freshly painted Iron work (Blood, 1979).
The obtained data that found in (Table 5) illustrated that, the Fe concentration , in condensed milk was ranged from 2.78 to 20.74 ppm with a mean value of 8.61 ± 1.14 ppm , while in evaporated milk it was ranged from 3.63 to 42.84 ppm with a mean value of 9.77 ± 1.94 ppm .lower level was reported by (Khalil and Seliem, 2013), while in infant formula the Fe concentration was ranged from 5.00 to 35.24 ppm with a mean value of 24.88 ± 1.77 ppm .this result are in agreement with (Iwegbuue, 2010) while higher level was reported by (Jolanta, 1996).
Table (5) & Fig (5) revealed that 20 (100%) of condensed and evaporated milk samples contained Fe above the recommended permissible limit (2.3 and 2.6ppm ppm, respectively) as established by (Holland *et al* ., 1995) and 20 (100%) of infant formula samples contained Fe within the recommended permissible limit (72ppm) as established by(European Commission, 1996) .
The high iron level in examined evaporated milk samples may be attributed to the fact that the chemistry involved in heavy metal delamination into canned food involves the following step: Detinning/corrosion, rusting of cans and leaching of the toxic metal from the corroded cans (Steve and Wallace, 2003).

Data represented in Table (6) illustrated that, Mn concentration , in condensed milk was ranged from 0.02 to 0.96 ppm with a mean value of 0.51 ± 0.07 ppm . lower level were reported by (Elena, 1999). On the other hand Mn concentration in evaporated milk was ranged from 0.03 to 1.50 ppm with a mean value of 0.47 ± 0.09 ppm . lower level were reported by (Elena, 1999) . In infant formula Mn concentration ranged from 0.13 to 2.03 ppm with a mean value of 0.76 ± 0.08 ppm . similar result was reported by(Jolanta, 1996) while higher level were reported by (Humaria *et al*., 2015) .
Table (6) & Fig (6) revealed that 12 (60%) of infant formula samples contained Mn above the recommended permissible limit (0.65 ppm) as established by (European Commission, 2006).

Manganese balance studies and excretion data indicate that low gastrointestinal absorption and rapid elimination of Mn limits the toxicity of the Mn following the ingestion of high dose (Barceloux, 1999) .

The results recorded in Table (7) & Fig (7) indicated that the average concentration of Pb, Cd,Cu,Zn,Fe and Mn in examined condensed milk samples were 880, 60, 5190, 9920, 8610 and 510 µg/kg , respectively . These concentrations gave a daily intake of about 88, 6, 519, 992, 861 and 51µg/person, respectively and these quantities representing 35.2, 8.6, 17.3, 6.61, 4.78 and 1.02 % of the acceptable daily intake recommended by (Codex Stand,1995) , (WHO, 1996), (IMNAS, 2000 and IMNAS and FNB, 2001) and (Perveen *et al*., 2005) .

The results recorded in Table (8) & Fig (8) indicated that the average concentration of Pb,Cd,Cu,Zn,Fe and Mn in examined evaporated milk samples were 1170, 70, 4520, 5560, 9770 and 470 µg/kg , respectively . These concentrations gave a daily intake of about 117, 7, 452, 556, 977 and 47µg/person, respectively and these quantities representing 46.8, 10, 15.06, 3.70, 5.43 and 0.94 % of the acceptable daily intake recommended by (Codex Stand 193-1995) , (WHO, 1996), (IMNAS, 2000 and IMNAS and FNB, 2001) and (Perveen *et al*., 2005) .

The results recorded in Table (9) & Fig (9) indicated that the average concentration of Pb, Cd, Cu, Zn, Fe and Mn in examined infant formula samples were 850, 100, 4460, 13420, 24880 and 760 µg/kg , respectively . These concentrations gave a daily intake of about 114.75, 13.5, 602.1, 1811.7, 3358.8

and 102.6 µg/infant, respectively and these quantities representing 318.75, 135, 12.04, 36.23, 22.39 and 17.1 % of the acceptable daily intake recommended by WHO ,1982 , 2000 and 2001) , (National Academy of Science, 2004) and (EFSA, 2009) . From aforementioned results, it could be concluded that the average daily intake of Pb, Cd in infant formula were relatively high.

Regarding the public health hazards of the detected metals, Lead does not break down in the environment and this potent neurotoxin can harm the nervous system, reproductive complications and kidney failure especially in young children (Miller *et al*. 2009).

Chronic Cd toxicosis included kidney damage with proteinuria, impaired regulation of calcium and phosphates, manifesting bone demineralization, osteomalacia and pathological fractures . Moreover, Cd is a possible cause of hypertension, insomnia and testicular atrophy (Friberg and Elinder , 1985) .

Copper is an essential trace element, as it is a component of many enzymes and Proteins in living organisms, required for normal growth of infants, host defence mechanisms, bone strength, maturation of erythrocytes and leukocytes, iron transport, and brain development (Schumann *et al*., 2002) . Excessive intake of copper has an irritating effect on the gastrointestinal tract and has previously been used to induce vomiting in the event of poisoning. High intakes of copper may in the long run lead to liver damage (Gabriela *et al*., 2013) .

Zinc is an essential constituent or cofactor for more than 200 mettalloenzymes, hormones and hormone receptors, protein, neuropeptides and polynucleotides *(*Lee, 1998) . Ingesting high levels of zinc for several months may cause anemia, damage the pancreas, and decrease levels of high-density lipoprotein (HDL) cholesterol (Tassew *et al* ., 2014) .

Excess Iron causes tissue damage by acting as a catalyst in the conversion of hydrogen peroxide to free-radical ions which attack cellular membranes, cause DNA strand breaks, inactivate enzymes, depolymerize polysaccharides and initiate lipid per oxidation (Freeman, 2004) .

Excess manganese interferes with the absorption of dietary iron. Long-term exposure to excess levels may result in iron-deficiency anemia. Increased manganese intake impairs the activity of copper metalo-enzymes (Blaurock –Busch, 2010).

From the previously mentioned results, it is evident that examined dairy products samples were contaminated with variable amounts of heavy metals. This amount in original milk is admittedly minute, but their contents may be significantly altered through manufacturing and packaging process where metal contamination may occur at several stages during dairy processing e.g. from factory door, plant equipment's, catering operations, ceramic, enameled utensils, metal containers and water used in dairy production (Reilly, 1991). So, it is necessary to control the manufacturing process at each step, in order to determine the source and levels of contamination and to ensure the desired product quality (Ayar *et al*., 2009).

In this sense, the determination of trace elements in food is of a great importance since the deficiency or excess of metals could promote several clinical disorders resulting public health problems .

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