CONCENTRATION OF TRACE ELEMENTS IN HUMAN AND ANIMAL MILK IN JEDDAH, SAUDI ARABIA

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SUMMARY: Human milk is the first food human encounter and it serves as the sole source of all nutrients required for the biological functions and growth during the early stages of life. Trace elements contents are therefore of importance from nutritional point of view. Moreover, accurate data on the concentrations of trace elements in human milk throughout early lactation are important for developing milk formula substitutes. Raw milk as it comes from cow is the natural substitute to human milk for infant feeding. It is now recognized that both too little and excessive amounts of minerals pose health hazards for the infants.

This study was directed to measure the concentrations of zinc (Zn), iron (Fe), copper (Cu), chromium (Cr), Cadmium (Cd), manganese (Mn) and lead (Pb) in human milk and in cow milk samples available in and around Jeddah city. A total of 108 human milk samples and 80 fresh cow milk samples were analyzed after wet digestion for the seven trace elements using Atomic Absorption Spectrometer. The mean concentrations of Zn, Cu and Fe in human milk are higher than the corresponding values in cow milk while the mean concentrations of Cr, Mn, Pb and Cd in human milk are lower than the corresponding values in cow milk. The concentrations of all elements in human milk collected in the afternoon are higher than those in samples collected in the morning. The experimental results show that there is an apparent decline in the mean elemental concentration levels as the stage of lactation progressed. Our results for human milk are also compared with the corresponding values of different countries available in literature.

Key Words: Zinc, iron, copper, chromium, cadmium, lead, manganese.

INTRODUCTION

Milk is the fundamental food for infants. The most natural and best source is from breast feeding and this is greatly encouraged for the first 6 months of life and should be continued for as long as 2 years. Human milk is also a source of immune agent which can, among other functions, hold intestinal disease in check - almost as important as nutrition itself (1, 2). Certain situations may warrant a special diet for metabolic reasons or in view of some pressing factors, artificial feeding may have to be restored too. For elite urban women, the ready - made milk preparations from the market have become handy for infant feeding. Raw milk as it comes from cow is the natural substitute to human milk for infant feeding. Powdered milk of different brands is available in a wide range to suit different ages. It is estimated that proper infant feeding can prevent millions of deaths occurring from infantile gastroenteritis and malnutrition (3,4).

With increasing environmental pollution a heavy metal exposure assessment study is necessary (3-5). Heavy metals enter human body through inhalation and ingestion. Intake via ingestion depends upon food habits. There is now growing evidence of the importance of trace elements in human nutrition, and there are reports that

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Table 1: Concentrations of Zn, Cu, Fe, Mn, Cd, Pb and Cr in standard reference milk sample (A-11) from IAEA.

Reference sample	Metal	Certified Concentration (IAEA Values) (µg/g)	Average observed concentrations (µg/g)
Milk sample IAEA (A-11)	Zn	38.9 (36.6-41.2)	37.1 (34.8-39.2)
	Cu	0.38 (0.35-0.41)	0.40 (0.37 - 0.42)
	Fe	2.1 (2.0-2.4)	2.2 (1.9-2.4)
	Mn	0.30 (0.26-0.32)	0.32 (0.29 - 0.34)
	Cd	1.7 (1.2-2.2)	1.6 (1.3 - 2.1)
	Pb	54 (29-79)	50.9 (48 - 55)
	Cr	0.27 (0.22-0.29)	0.25 (0.24 - 0.28)

suggesting trace elements deficiencies can lead to impaired growth during infancy and childhood (1,2). Since the neonatal period is one of the most critical with respect to nutrition, there is need to know the actual intakes of trace elements by fully breast feeding infants during the 1st month postpartum. It is well established that Pb and Cd are toxic and children are more sensitive to these metals than the adults. The metals namely Fe, Cu and Zn are essential micronutrients and have a variety of biochemical functions in all living organisms. In recent years this has led scientists to examine the trace elements content of human milk, the ideal infant food during the first month of life, in order to estimate infant requirements and establish reference values for use in manufacturing infant formulas. While Fe, Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from elements-to-elements (3,4,6). Information on the secretion of trace elements in human milk is needed, not only in order to estimate the intake by breast-fed infant, but also as a starting point for recommendations for intakes from other types of infant foods and maternal diets during lactation.

The aim of the present study is to determine the concentrations of some trace elements, namely Zn, Cd, Cr, Cu, Fe, Mn and Pb in mother milk and animal milk available in and around Jeddah city. A total of 108 samples of human milk and 80 samples of animal milk (fresh cow milk) were analyzed after 'wet digestion' for seven trace elements using Graphite Atomic Absorption Spectrometer (AAS). The obtained metal concentrations are compared with the corresponding values of different countries available in the literature.

MATERIALS AND METHODS

Collection of milk samples

For the present study, commercially available fresh cow milk samples of 11 different brands were collected from different supermarkets in Jeddah city. An amount of 1 liter milk in a paper card board/plastic bottle was collected for each sample. A total of 80 samples were analyzed.

Table 2: Elemental concentration in human milk.

Element	Mean Concentration (ppb)		Mean concentration of morning and afternoon samples (ppb)	Range (ppb) (min-max)	
	Morning samples	Afternoon samples		Morning samples	Afternoon samples
Zinc	1292.6 ± 1.5	1448.3 ± 1.7	1370.5 ± 1.1	1004.5 - 1878.0	1008.8 - 2141.3
Copper	612.2 ± 0.5	634.6 ± 0.7	623.4 ± 0.4	51.7 - 1030.0	72.3 - 1099.3
Iron	303.1 ± 0.6	359.9 ± 0.6	331.5 ± 0.4	186.3 - 596.0	220.3 - 625.0
Chromium	15.2 ± 0.2	16.7 ± 0.2	16.0 ± 0.1	5.1 - 33.0	5.0 - 38.0
Manganese	4.6 ± 0.1	5.8 ± 0.1	5.2 ± 0.07	1.0 - 9.3	2.0 - 14.7
Lead	3.5 ± 0.0	4.3 ± 0.0	3.9 ± 0.0	1.0 - 14.7	1.7 - 13.7
Cadmium	1.6 ± 0.0	2.1 ± 0.0	1.9 ± 0.0	1.0 - 13.0	1.0 - 13.1

Table 3: Elemental concentration in cow milk.

Type of milk	Brand name	Concentration in ppb						
		Zinc	Iron	Copper	Chromium	Manganese	Cadmium	Lead
Fresh cow milk	Al-Safi	599.2 ± 6.7	319.6 ± 5.8	51.1 ± 2.0	30.2 ± 1.3	18.2 ± 1.2	1.0 ± 0.0	1.1 ± 0.1
	Al-Marai	253.3 ± 5.2	307.3 ± 5.2	53.0 ± 1.3	22.0 ± 0.8	20.4 ± 1.9	2.0 ± 0.2	1.1 ± 0.2
	Al-Hanah	1115.1 ± 8.6	338.4 ± 6.7	53.1 ± 2.1	12.1 ± 0.9	18.5 ± 1.3	4.1 ± 0.5	3.2 ± 0.4
	KAA University	1144.3 ± 8.9	464.5 ± 6.1	43.1 ± 1.7	58.6 ± 1.8	25.7 ± 1.8	2.0 ± 0.4	1.0 ± 0.0
	King Saud University	1180.0 ± 9.2	592.7 ± 9.4	27.2 ± 1.1	24.2 ± 1.3	14.4 ± 1.3	5.1 ± 0.6	1.0 ± 0.1
	Gassim (Gassim Area)	990.2 ± 7.3	464.1 ± 6.1	41.0 ± 1.6	14.1 ± 1.0	16.3 ± 1.4	5.1 ± 0.8	5.2 ± 1.0
	Gassim (Burayda Area)	1235.2 ± 9.3	299.8 ± 6.5	80.1 ± 2.7	30.3 ± 1.2	21.5 ± 1.7	1.0 ± 0.4	4.3 ± 0.9
	Najyah	1046.3 ± 8.5	300.1 ± 7.3	37.0 ± 1.3	18.3 ± 1.1	21.6 ± 1.6	18.2 ± 1.3	5.2 ± 1.2
	Nadec	623.2 ± 5.8	288.2 ± 7.6	23.1 ± 1.0	28.4 ± 1.3	8.4 ± 0.9	7.2 ± 0.7	0.0 ± 0.0
	Tabiah	908.4 ± 6.1	783.5 ± 8.5	42.0 ± 1.4	48.6 ± 1.7	13.3 ± 1.1	2.0 ± 0.3	1.0 ± 0.2
	Assafwa	1200.0 ± 2.4	612.4 ± 9.1	88.1 ± 3.1	58.6 ± 1.9	15.6 ± 1.0	4.1 ± 0.6	15.2 ± 1.6
Mean		944.9 ± 2.4	413.5 ± 2.1	48.9 ± 0.6	31.4 ± 0.4	17.2 ± 0.4	4.7 ± 0.2	3.5 ± 0.2

The human milk samples were collected from lactating mothers in King Abdulaziz University Hospital, Jeddah. A doctor and a nurse were assigned to collect human milk samples. The human milk samples were collected twice in a day - one in the morning before breakfast and feeding and second sample in the afternoon after lunch before feeding to investigate the effect of diet on the elemental concentration for the same subject. We used sterilized plastic bottles to collect human milk samples. All milk samples were put in the freezer until the analysis was carried out.

The women who gave oral concent were enrolled during the first week postpartum. A total of 42 lactating mothers were selected. Among these, 35 mothers lived in urban area, while 7 lived in rural areas. Of the total, 19 of mothers were from low-income, 21 from middle-income and 2 from high-income families. The mean age of the mothers was 26 years (ages: 21-40). All the mothers were healthy and apparently well nourished women, based on clinical observation and had no history of any serious disease. All had uncomplicated pregnancies and delivered a single full term infant (39 ± 2 weeks). All infants were healthy and growing well. The mean birth weight of the infants was 3285 g (range: 2612 - 4300 g). Most of the milk samples were collected from day 1 (day of delivery) to 3 days postpartum. Few samples were collected from day 1 (day of delivery) to 7 days postpartum. The protocol for milk collection was designed to minimize trace elements contamination. The breast was cleaned with de-ionized water and breast milk was hand expressed, into 30 ml trace elements free polystyrene flask. Milk samples were transported to the laboratory on ice. Aliquots of the milk were transferred into 15 ml trace elements free polystyrene tubes, and then frozen to -20 ºC until analysis. The entire collection procedure was checked for copper, iron, zinc and manganese and was found to be free from contamination.

Reagents and glass wares

Atomic Absorption spectroscopic standard solutions for Zn, Cd, Cr, Cu, Fe, Mn and Pb were purchased from Fisher Scientific Company, USA. Working standard solutions were prepared by diluting the stock solution. Sulfuric acid, perchloric acid and nitric acid were all of AR quality (BDH, England). All glass wares (Conical flask, volumetric flask, watch glass, pipette, measuring cylinder, etc.) were of borocylicate (England). De-ionized water has been used where required.

Sample digestion and preparation of analyte solution for AAS

The milk sample needs to be brought into clear solution for analysis by Atomic Absorption Spectrometer. For this reason the milk sample was first digested with chemicals where the organic matrix of milk was destroyed and left the element into a clear solution. ‘Wet Digestion’ method (i.e. digestion with nitric, sulfuric and perchloric acids) has been used in the present study. The detailed procedure is available in the literature (7-9).

Figure 1: Concentration versus Absorbance calibration curves for Fe and Cu.

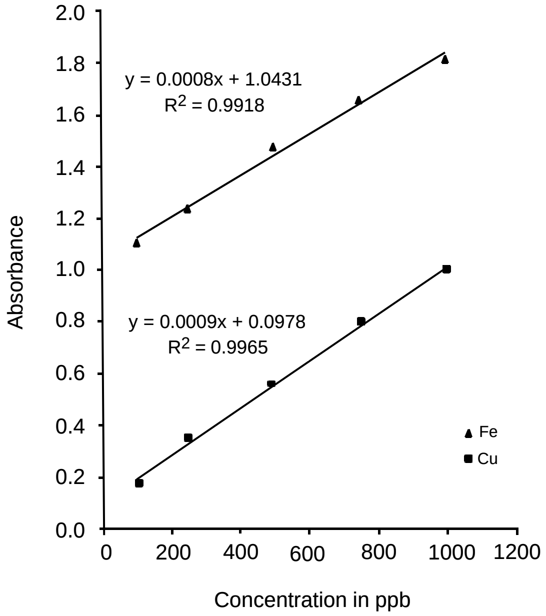
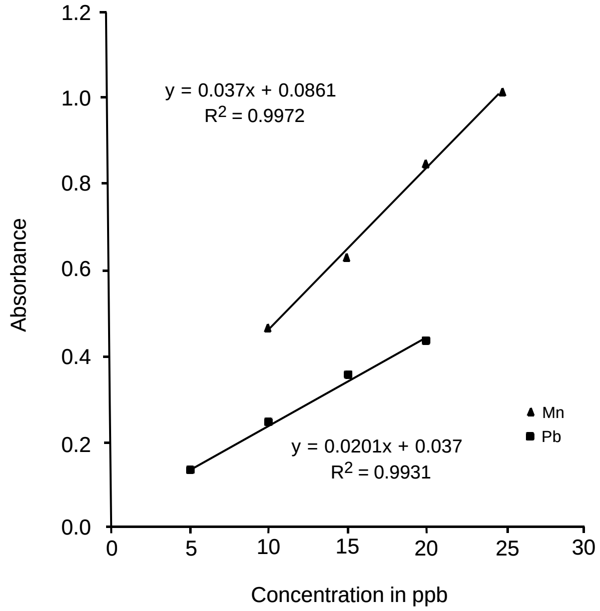


Figure 2: Concentration versus Absorbance calibration curves for Mn and Pb.



Calibration curve

The VARIAN Atomic Absorption Spectrometer (AAS), Model spectra AA 30P consisting of a double beam, four lamp Turrent Spectrometer with a Deuterium background corrector and a temperature programmable Graphite Tube Furnace Assembly (GTA 96) was used in this study. Temperature program of the furnace was optimized to obtain the best signal during the atomization process. Drying time and ashing temperature for each element was determined earlier. All these data were fed into the computer associated with the AAS machine. The range of linearity of the concentration vs. absorbance curve is of great importance in determining the elemental concentration of the milk samples. Standard aqueous solutions of different elements obtained from Fisher Scientific Company, USA were used to calibrate the AAS machine. The calibration curves were drawn for Zn, Cd, Cr, Cu, Fe, Mn and Pb by Machintosh Microcomputer using linear regression analysis of the concentrations of the standard solutions versus absorbance values.

A new calibration curve was plotted for each element every time a new batch of milk was arranged for analysis. Each standard solution was measured at least three times and the mean was plotted.

The sensitivity of the AAS machine was tested by using 10 ppb standard lead (Pb) solution. The mean absorbance value of several measurements was found to agree well with the manufacturer's stated value with a relative standard deviation (RSD) of 1.6%.

Measurement of elemental concentration in milk samples

10 µl aliquot of each milk sample, obtained after wet digestion, was injected into the graphite tube of the AAS with the help of an auto-sampler, and the elemental concentration was read from the output of the printer connected to the computer. Each sample was repeated several times for each element and the average was recorded. The concentrations of Zn, Cd, Cr, Cu, Fe, Mn and Pb were determined for each sample of human milk and animal milk. A total of 80 samples of fresh cow milk and 108 samples of human milk were analyzed in the present study.

Quality control

The reliability of method for estimation of Zn, Cd, Cr, Cu, Fe, Mn and Pb concentration in milk samples by AAS technique has been checked by analyzing standard reference milk sample (A-11) obtained from the International Atomic Energy Agency (IAEA). Measurements were taken several times and the average result agreed within ±7% of the certified values. Our values agree well with values published by different authors (3,10,11).

RESULTS AND DISCUSSION

The results of our measurements of the elemental concentrations in standard reference milk sample (A-11) obtained from IAEA are presented in Table 1.

The range of linearity of concentration versus absorbance graph is of great importance in determining the elemental concentration of the milk samples. The calibration graphs obtained for Fe, Cu, Mn and Pb are shown in Figures 1 and 2. Similar graphs were also drawn for Zn, Cd and Cr but are not shown here.

The adjusted linear equations and correlation coefficients are:

Iron	y = 0.0008x + 1.0431, r = 0.9959
Copper	y= 0.0009x + 0.0978, r = 0.9982
Manganese	y = 0.037x + 0.0861, r = 0.9986
Lead	y = 0.0201x + 0.037, r = 0.9965
Zinc	y = 0.0005x + 2.233, r = 0.9919
Chromium	y = 0.0054x, r = 0,9997
Cadmium	y = 0.0569x + 0.121, r = 0.9879

The observed correlation coefficients were also assessed using student's t-test at 5% level of significance. The results showed that there is significant correlation between the variables for each element under study.

The results of the present study for seven trace elements in human milk samples are given in Table 2. The mean of 40 milk samples collected in the morning and the mean of 40 milk samples collected in the afternoon are presented. In both types of samples, the mean concentration of zinc is the highest followed by copper, iron, chromium, manganese, lead and the last is cadmium. It is clearly evident from Table 2 that the concentrations of all the elements in the afternoon samples are higher than those in the morning samples. Feeley *et al.* (12) also reported that elemental contents of the AM samples were significantly lower than those of the PM samples.

Figure 3: Concentration of zinc in human milk from 1 to 7 days postpartum.

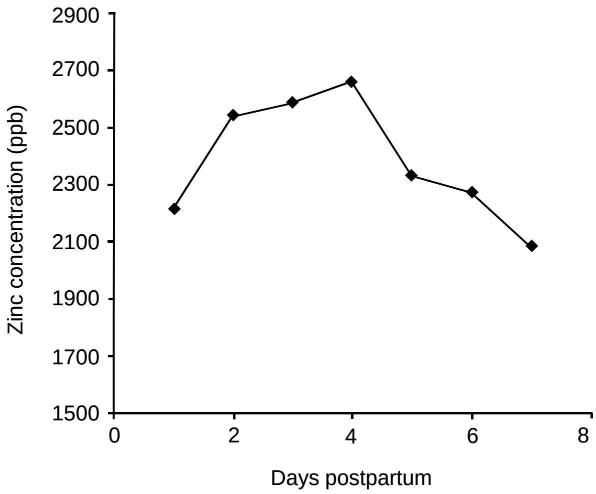


Table 4: Comparison of the elemental concentrations in human milk samples of different countries.

Country	Concentration (µg/l)							Reference
	Cr	Cu	Zn	Fe	Mn	Cd	Pb	
USA	-	200-900	500-5000	-	-	-	-	Michael (1983)
Germany	-	350	2300	-	-	-	-	Bratter *et al.* (1987)
Sweden	-	-	-	-	-	0.1	2.0	Larsson *et al.* (1988)
Sweden	-	-	-	-	-	0.6	0.7	Hallen *et al.* (1995)
55 Countries (Mean value)	-	29	1680	-	-	1.3	-	Woittiez and Iyengar (1988)
Germany	-	270	1400	-	-	-	-	Jachum *et al.* (1995)
Sudan	-	117	1300	-	-	-	2.6	AbuSamara (1995)
India	-	195	1772	-	-	0.09	1.9	Tripathi *et al.* (1999)
Bangladesh	-	120-250	280-1800	330-700	-	-	-	Khan *et al.* (1989)
Poland	14.2	1100	5017	-	-	6.2	5.4	Baranowska (1994)
USA	-	1009	5067	937	-	-	-	Feeley *et al.* (1983)
Spain	12.4-22.3	210-1191	-	237-860	5.9-22.8	-	-	Mingorance and Lachica (1985)
Cited values	6.4-18.5	30-750	-	260-730	4.8-17.8	-	-	Mingorance and Lachica (1985)
Saudi Arabia	16.1	629.6	1384.2	334.8	5.2	1.9	3.9	Present Study

Table 5: Comparison of the elemental concentrations in cow milk of different countries.

Country	Concentration (µg/kg)							Reference
	Cr	Cu	Zn	Fe	Mn	Cd	Pb	
Japan	-	100	3000	-	-	1	50	Cortes *et al.* (1994)
Germany	-	36.6	3390	-	-	0.1	1.6	Ostapczuk *et al.* (1987)
Germany	-	30	1909	-	-	-	-	Jachum *et al.* (1995)
India	35.2	39.3	2888	-	-	0.1	1.6	Krishnamoorthy and Tripathi (1998)
Spain	33.9	50.7	1419.4	997.3	26.4	-	9.3	Schuhmacher *et al.* (1993); Mingorance and Lachica (1985)
Poland	20-40	42-47	3000-3910	180-740	10-40	3-20	22-25	Bulinski *et al.* (1992)
USA	29.1	19.4	2335.2	-	50	9.7	14	Lopez *et al.* (1985); Khan *et al.* (1989)
Bangladesh	-	97.2	1214.5	577.2	-	-	-	Khan *et al.* (1989)
Saudi Arabia	31.4	48.9	944.9	413.5	17.2	4.7	3.5	Present Study

We managed to collect human milk samples from few lactating mothers from 1 to 7 days postpartum. The mean concentrations of different elements were plotted as a function of the collection day of milk samples in Figures 3-5. The variation of Fe, Mn, and Pb are not shown here. It is observed that there are large and apparently irregular changes occurring in the early postpartum period. There is an apparent decline in the mean levels as the stage of lactation progressed. The declination in trace elements concentrations in human milk as the stage of lactation progressed has also been reported by different authors (13-16).

The results of the study for seven trace elements: Zn, Fe, Cu, Cr, Mn, Pb and Cd in 80 fresh cow milk samples of different brands are presented in Table 3. The mean concentration of Zn is the highest followed by Fe, Cu, Cr, Mn, Cd and the last Pb. The distribution of concentrations of seven trace elements in human milk and cow milk are shown in Figure 6. The mean concentrations of Zn, Cu and Fe in human milk are higher than the corresponding values in cow milk while the mean concentrations of Cr, Mn, Pb and Cd in human milk are lower than the corresponding values in cow milk.

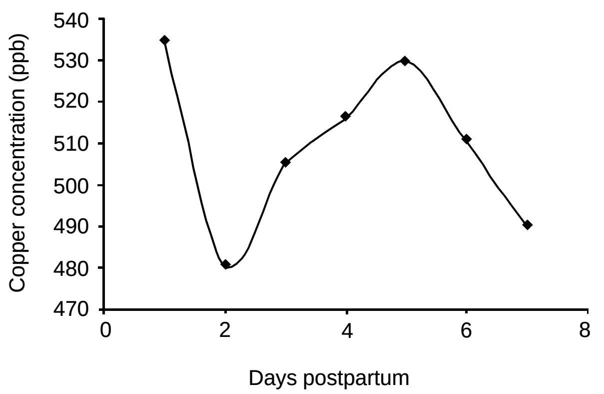


Figure 4: Concentration of copper in human milk from 1 to 7 days postpartum.

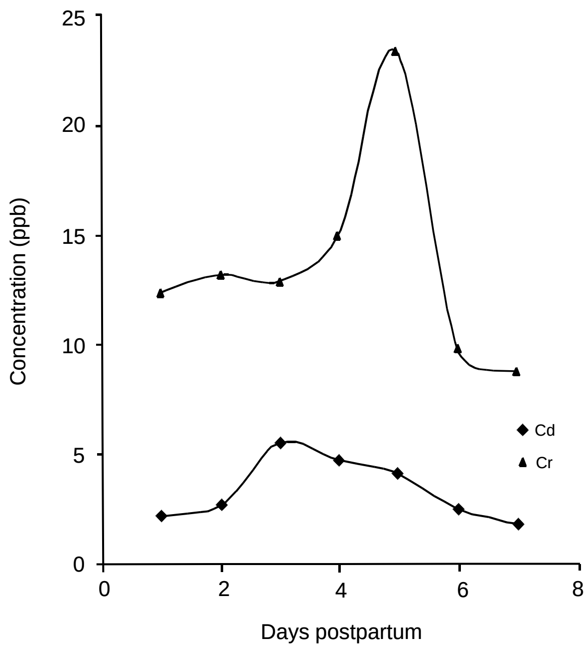
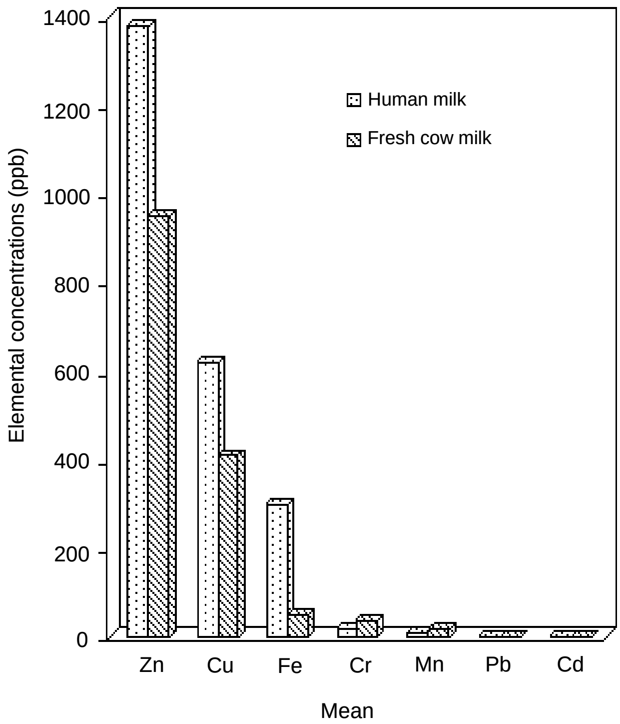


Figure 5: Concentrations of cadmium and Chromium in human milk from 1 to 7 days postpartum.

Table 6: Comparison of daily intakes of metals from 500 ml of breast milk by Saudi infants with recommended values.

Elements	Daily intake from breast milk (μg/day)	Recommended tolerable value (μg/day)	Reference
Cr	8.0	10-120	Mohan and Stump (2000) Casey *et al.* (1985)
Cu	314.8	500-1000	Tripathi *et al.* (1999) Mohan and Stump (2000)
Zn	692.1	3000-5000	Tripathi *et al.* (1999) Mohan and Stump (2000)
Fe	167.4	5000-6000	Mohan and Stump (2000) Feeley *et al.* (1983)
Mn	2.6	500-700	Mohan and Stump (2000) Casey *et al.* (1985)
Cd	0.9	2.8-3.5	Tripathi *et al.* (1999) Vijaya (1993)
Pb	1.9	12.5-17.5	Tripathi *et al.* (1999) Vijaya (1993)

Figure 6: Comparison of mean elemental concentrations in milk samples from two sources, human milk and fresh cow milk.



There is wide variations in the published data for the elemental concentrations of human milk of different countries (3,6,7,12,17-22). Some of the results are recorded in Table 4 for comparison with the present values. The concentration of Zn in Saudi Arabian mother milk is found to be the lowest, however, similar ranges, in fact lower concentration values have been reported in Sudan, Bangladesh and Germany (17,19,20). Although the concentration of Cu is high in breast milk in Saudi Arabia, this level is much lower compared to the values observed in western countries such as Poland and USA (7,12). Similarly the concentrations of Cd and Pb are higher in breast milk in Saudi Arabia but these values are lower compared to the values observed in Poland (7). The concentration levels of Fe, Mn and Cr in breast milk observed in this study are well comparable with those reported elsewhere (21,22).

There are also wide variations in the published data for the elemental concentrations of cow milk of different countries (3,19,20,23-28). Some of the results are recorded in Table 5 for comparison with present values. The Cr and Cu concentrations of the present study are well comparable with the published data. But the present values of Zn, Fe, Mn and Pb concentrations are very low compared with the corresponding values of other countries.

The daily intake of the metals depends on both the concentration and the amount of food consumed. The only food of breast feed infants (age less than 1 month) is the human milk. The mean of 24 hours output of breast milk of an Indian mother is 550 ml for first 3 months, 680 ml for 4 to 6 months and 700 ml for 6 to 12 months (3,29,30). According to results published by Feeley et al. (12), the mean daily outputs of breast milk of an American mother are 400, 500 and 600 ml at the early transitional and mature stages of lactation, respectively.

Assuming a value of 500 ml/day for the milk consumption by the infants in Saudi Arabia, the daily intake of these metals by Saudi infants are determined and are depicted in Table 6. The Recommended Dietary Allowance (RDA)/Tolerable/Recommended Safe and Adequate value as set by different international organizations, such as NCR and FAO/WHO are also given in the same table. It is evident that the intakes of essential elements (Zn, Cu, Fe, Mn and

Cr) are well below the recommended/tolerable values but the breast milk of Saudi mothers is very poor source of Zn, Fe and Mn. The intake of toxic elements Cd and Pb are also well below the recommended/tolerable levels (3,31).

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