A comparative study of trace elements in human, animal and commercial milk samples in Erbil, Iraq

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Abstract

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. However, overdose of these mineral constituents can be harmful. This study was directed to measure the concentrations of Zinc (Zn), Cadmium (Cd), Chromium (Cr), Copper (Cu) cobalt (Co), nickel (Ni), iron (Fe) and manganese (Mn) in human, animal, commercial fresh cow milk samples available in Erbil city. A total of 30 human milk samples, 10 animal milk samples (cow and goat) and 27 commercial fresh cow milk samples were analyzed after wet digestion for the eight trace elements using Atomic Absorption Spectrometer. The mean values of contents (in ppb) of these elements in breast milk obtained from mothers 1~3 days postpartum were 2.47, 6.56, 18.93, 5.04, 564.29, 1163.47, 302.15 and 6.94, respectively. While those in animal milk were 3.375, 7.75, 14.5, 28, 300.5, 1012.5, 183 and 4.23, respectively. And those in commercial fresh cow milk were 4.51, 9.044, 19.55, 17.65, 395.09, 841.211, 35.72 and 6.95, respectively. The mean concentrations of these elements in breast milk comparable with values of WHO/IAEA study.

الخلاصة

يعتبر حليب الانسان الغذاءِ الأولِ الإنسانيِ وهو يَعْملُ كالمصدرِ الوحيدِ لكُلّ المواد المغذّية المتطلبة للوظائفِ والنمو الحيويِ أثناء المراحلِ المبكّرةِ للحياةِ. محتويات العناصرِ النزرة لها اهميةِ مِنْ وجهةِ النظر المغذّيةِ. علاوة على ذلك،هناك بيانات دقيقة لتراكيز العناصرِ النزرة في الحليبِ الإنسانيِ بالفترة المبكرة للإرضاعِ تعتبر مهمة لبدائلِ صيغةِ الحليبِ المحتاج للنمو.ان الحليب الخام كما يَجيءُ مِنْ البقرة ِفهو البديل الطبيعيُ إلى الحليبِ الإنسانيِ لإطعام الرضيع.

إن الزيادة أو النقصان لبعض العناصر المتواجدة في الحليب بكميات ضئيلة تؤدي إلى ظهور بعض الأعراض الجانبية. في هذا البحث تم قياس تركيز بعضًا من هذه العناصر مثل الكروم، النحاس، الزنك، الكادميوم، الحديد ،الكوبلت ، المنغنيز ، والنيكل المتواجدة في حليب الانسان وحليب الحيوان(البقرة والعنزة) والحليب التجاري

المتواجد في اسواق مدينة اربيل بكميات ضئيلة. ان مجموع 30 نموذج من حليب الانسان،10 نماذج من حليب الحيوان (البقرة والعنزة)،27 نموذج من الحليب التجاري قد تم قياسه بعد عملية هضم البروتين للثمانية العناصرِ النزرة وذلك باستخدام جهاز مطياف الامتصاص الذري . وكانت متوسط القيم لمحتويات هذه العناصر(بوحدة جزء من بليون جزء) في عينات حليب صدر الام المأخوذ من الامهات من (1 ~3) ايام بعد الولادة ، وكانت كالتالي : 2.47، 6.56، 18.93، 5.04، 564.29، 1163.47، 302.15 و 6.94 على التوالي. بينما متوسط القيم لمحتويات هذه العناصر في الحليبِ الحيواني المأخوذ من البقرة والعنزة كَانتْ 3.375، 7.75، 14.5، 28، 300,5 ، 1012.5، 183 و 4.23 على التوالي. وأولئك في حليبِ البقرةِ الطازجِ التجاريِ كَانتْ 4.51، 9.044، 19.55، 17.65، 395.09، 841.211، 35.72 و6.95 على التوالي. وقد تمت مقارنة متوسط تراكيز هذه العناصرِ في نموذج حليبِ صدرِ الام (الانساني) مع قيم الدراسة التي تمت من قبل بعض منظمات WHO/ IAEI.

Introduction

Milk is one of the important foods for human nutrition that's why determination of trace element contents is increasing importance since last decades. (1)
In recent years, quite sensitive analytical techniques such as polarized atomic absorption spectrophotometer have been developed for the determination of trace elements. Although a number of studies have been reported on essential trace elements such as zinc, copper, and iron of milk,(2) only limited data are available for the contents of cobalt, nickel, chromium, manganese, and molybdenum, which have been also recognized as essential for the growth of living organisms. (3-5)
With increasing environmental pollution a heavy metal exposure assessment study is necessary. (6-8) 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 suggesting trace elements deficiencies can lead to impaired growth during infancy and childhood. (9,10) 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. (7- 11)
The aim of the present study is to make a comparative study of the availability of trace elements; human and animal (cow, goat) milk and commercial fresh cow milk for 8 trace (Mn, Fe, Cu, Zn, Co, Cd, Cr and Ni) elements have been analyzed using Atomic Absorption Spectrometer (AAS). The obtained metal concentrations in breast milk are compared with the values of WHO/IAEA study.

Experimental procedure

Collection of milk samples

For the present study, commercially available fresh cow milk samples of 9 different brands were collected from different supermarkets in Erbil city. An amount of 1 liter milk in a paper card board/plastic bottle was collected for each sample. Animal milk samples from single species of cow and goat were collected. The human milk samples were collected from lactating mothers in Gymea Obstetrical Hospital, Erbil city. The human milk samples were collected in the morning before breakfast and feeding to investigate the effect of diet on the elemental concentration for the same subject. All milk samples were put in the freezer until the analysis was carried out. A total of 10 lactating mothers were selected. The mean age of the mothers was 28 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. The milk samples were collected from day 1 (day of delivery) to 3 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 polystyrene flask. Milk samples were transported to the laboratory on ice, and then frozen to -20^{o}C until analysis. The entire collection procedure was checked for copper, iron, zinc and manganese and was found to be free from contamination.

Apparatus and Reagents

Atomic Absorption spectroscopic standard solutions for Zn, Cd, Co, Cr, Cu, Fe, Mn and Ni 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 borosilicate (England). De-ionized water has been used where required.

Sample Digestion and Preparation of Analytic Solution for AAS

The milk sample needs to be brought into clear solution for analysis by the 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. [8]

Measurement of elemental concentration in milk samples

Aliquot of milk samples 10µl obtained after wet digestion was injected into the tube of the AAS. Each sample was repeated three times for each element. At least three milk samples of each brand collected at different times of the year were analyzed for each element. The concentrations of Zn, Cd, Co, Cr, Cu, Fe, Mn and Ni were determined for each sample.

Calibration Curve

The Atomic Absorption Spectrometer (AAS), Model Spectra AA 30 P consisting of a double beam, was used in this study. 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, Co, Cr, Cu, Fe, Mn and Ni 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 samples 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 zinc (Zn) solution. The mean absorbance value of several measurements was found to agree well with the manufacturer's stated value.

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 Cr are shown in Figures 1 and 2. Similar graphs were also drawn for Zn, Cd, Co and Ni but are not shown here.

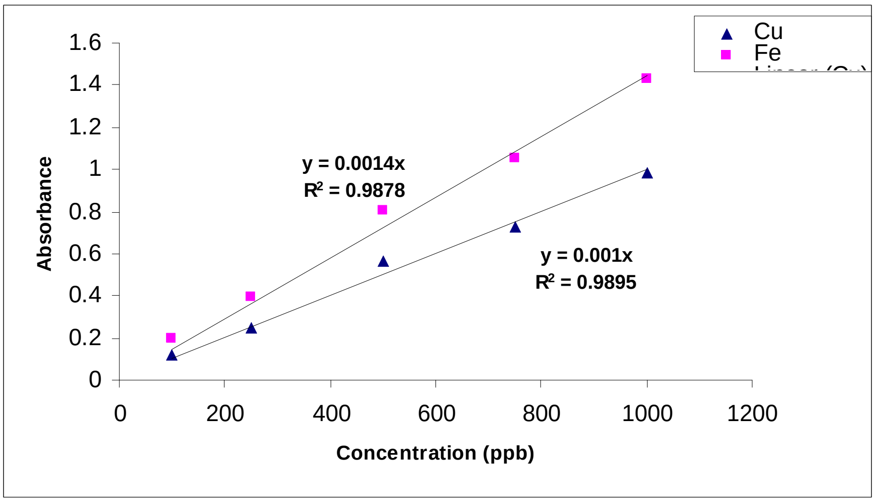

Figure 1: Concentration versus Absorbance calibration curves for Fe and Cu.

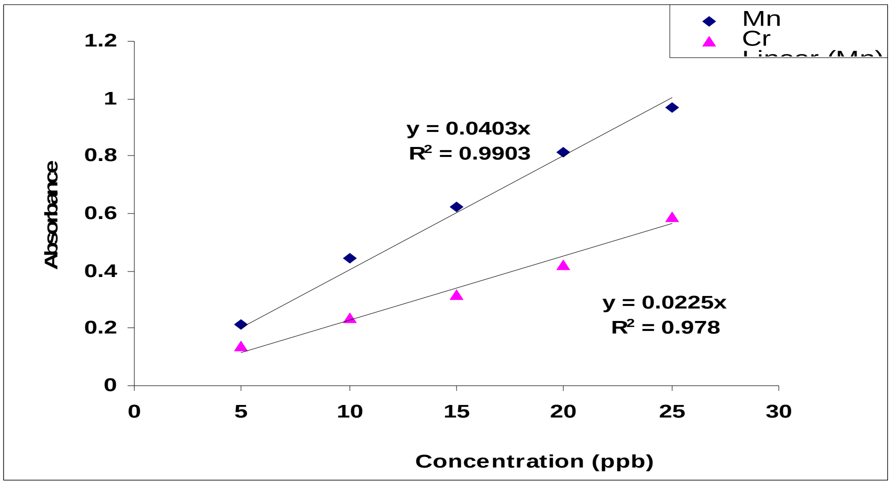

Figure 2: Concentration versus Absorbance calibration curves for Mn and Cr.

Results and discussions

Contents of trace elements in breast milk (human milk)

Trace minerals are necessary components of the human diet. However, only small amounts of these elements are needed to carry out the necessary biological reactions. [12] Metals do not accumulate in fat, and so do not usually achieve higher concentrations in breast milk than in blood. As a result, infants are likely to be exposed to higher levels before birth than during breastfeeding. Nonetheless, learning about metals in breast milk is important for two reasons: first, as a pathway of exposure, and second, as an indicator of likely prenatal exposures. [13] The eight trace elements determined in this investigation, namely: cobalt, nickel, chromium, manganese, iron, cadmium, zinc and copper are considered essential for the growth of living organisms. The concentration of these elements in breast milk is presented in (Table 1).The mean of 30 milk samples collected in the morning are presented for different ages of mothers (ages: 21-40) .The values of the elements were being different from age to another, may be according to the nutrition of mothers. The mean concentration of zinc is the highest followed by iron, copper, chromium, cadmium, nickel, manganese and the last is cobalt. The mean concentrations of nickel, chromium and cadmium compare excellently with those of a WHO/IAEA study [14] from five countries (Hungary, Nigeria, Philippines, Sweden and Zaire) (Table2). For other elements such as manganese, iron, zinc and copper, the values are lower than it. Dang et al.[15-17] have reported the analysis of Fe, Co, Se, As, Mn, Cu and Zn in Indian mother's milk from Bombay. It is observed that only Fe and Zn concentrations are comparable, while other elemental contents are higher. In this study Zn, Cu, Fe and Mn concentrations are also with in comparable range of an exhaustive study of American mother's milk by Casey et al. [18] It has been suggested that the composition of human milk is never constant and several factors such as geographical location, age, environmental aspects, stage of lactation and overall nutritional status of mother are responsible for the observed elemental variations. The average value of cobalt was 2.47 ng per g of samples (Table 1). This was similar to 2.0 ng per g (range 1.3~3.0 ng per g) reported by Grimanis et al. [19] The average value of chromium in this study was 18.93 ng per g of samples (Table 1). This was higher than to the value of 4.6~6.4 ng per g reported by Krishnamachari [20], but similar to the value of 18-40 ng per g of milk sample reported by Grimanis et al. [19] Casey [21] reported 20ng/ml nickel of breast milk obtained from mothers who have been lactating for 4 to10 days. The study showed 6.50 ng nickels per g of milk samples, which is similar to 6.56 ng per g of samples in this study (Table1).

Table 1: Mean concentration in (ppb) of trace elements in human milk sample*.

Age of mother	Co	Ni	Cr	Mn	Fe	Zn	Cu	Cd
21	8.5	1.5	10.3	1.0	134.6	1014.9	191.8	10
25	2.3	3.6	33.8	2.7	412.5	1292.6	339.8	3.5
26	1.1	6.3	16.4	10.5	718.9	1095.7	421.7	8.4
27	1.3	0.9	14.7	1.8	623.5	1004.2	330.2	1.2
29	2.9	7.3	7.1	5.2	820.1	1149.5	331.8	13.2
31	1.0	7.6	4.5	2.1	126.7	1436.3	228.4	1.9
33	0.6	9.6	40.2	3.2	589.2	1025.7	186.5	9.7
35	1.4	12.4	30.5	7.8	242.4	1336.2	218.2	1.8
38	4.7	5.8	9.3	6.3	935.1	1187.6	590.5	4.7
40	0.9	10.6	22.5	9.8	1039.9	1092.0	182.6	15.0
mean	2.47	6.56	18.93	5.04	564.29	1163.47	302.15	6.94

**Concentration values are reported with respect to evaporated milk. Each entry is the mean of three aliquots of human milk.*

Table 2: Comparison of the mean concentrations of eight trace elements of human milk in Iraq with the values of WHO/IAEA study. (14)

Elements	The present study	WHO/IAEA study
Co	2.47	1.5
Ni	6.56	7.02
Cr	18.93	19
Mn	5.04	72
Fe	564.29	1950
Zn	1163.47	1210
Cu	302.15	1600
Cd	6.94	6.46

Contents of trace elements in animal milk (cow & goat)

Amongst all the animal milk samples, cow milk has been the most widely analyzed. (22-26) In South-East Asian countries, goat milks are also consumed on a large scale.

Iyengar (27) reviewed the elemental composition of human and animal milk. The main components of goat milk are similar to those of cow milk but differs as to particular physical and chemical properties (small size of fat globules, higher content of short and medium chain fatty acids. (28) It is noteworthy that milk of each species has a particular individual pattern of minerals, which may be a pointer of the relative nutritional importance of the element.(29)

In present study concentrations of trace elements in animal milk (cow and goat) are shown in (Table 3).

Cow milk is distinguished by a lower concentration of Fe with goat milk type, while the mean concentration of other trace elements (Zn, Cd, Cr, Cu, Mn and Ni) are being higher in cow milk. The mean cobalt level for a mixture (cow and goat)samples was 3.37 ng per g (Table 3). This was similar to 3.0 ng per g (range 1.3~3.9 ng per g) reported by Hiromi et al. (30) but some what higher than those of (0.3~1.1 ng per g) reported by Titova and Derzhavina. (31) The mean nickel level for a mixture (cow and goat) milk samples was 7.75 ng per g (Table 3). This is in good agreement with those reported by Mitchell (32) and Casey, (21) but other reports showed considerably higher values ranged from 20 to 50 ppb. (33, 34) .These higher values may be due to contamination, since nickel could easily contaminate milk from stainless steel used for milking machines, metal containers, and pipes. The mean chromium and manganese level for a mixture (cow and goat) milk samples was (14.5 and 28 ng per g) (Table 3). This is in good agreement with values (14 and 27 ng per g) those reported by Hiromi et al. (30)

Contents of trace elements in commercials milk

With increasing industrialization and technological advancement, fresh milk has been rapidly replacing natural milk almost everywhere. Several different brands of formula milk powders are available around the world for infants and adults with added nutrients (as per manufacturer's claims).(35) In this study nine different brands of commercial fresh cow milk from the Erbil market has been analyzed. In (Table 4) are listed the trace elemental concentrations in different brands of commercial fresh cows milk with mean values for the three categories. The mean concentrations of zinc are the highest followed by iron, copper, chromium, manganese, nickel, cadmium and the last is cobalt.

Since these samples are based on cow milk, it is not surprising that contents of cobalt, nickel, chromium and cadmium in ppb commercial milk samples are similar to those in cow milk (Table 3and 4).while contents of manganese, zinc and copper in ppb commercial milk samples are apparently lower than those in cow milk (Table 3and 4).Only the mean concentration of iron in commercial milk sample are higher than those in cow milk (Table 3and 4). Kinsara and Farid [36] have reported the analysis of Fe, Cd, Cr, Mn, Cu, Co, Pb and Zn in human milk and in cow milk samples available in and around Jeddah city in Saudi Arabia. The values of there study are nearly to the values of concentration of the trace elements of the present study.

Table 3: Mean concentration in (ppb) of trace elements in animal milk (cow and goat) sample*.

Samples of raw milk	Co	Ni	Cr	Mn	Fe	Zn	Cu	Cd
Cow	4.45	8.7	18	32	271	1080	190	4.85
Goat	2.3	6.8	11	24	330	945	176	3.61
mean	3.375	7.75	14.5	28	300.5	1012.5	183	4.23

**Concentration values are reported with respect to evaporated milk. Each entry is the mean of three aliquots of animal milk.*

Table 4: Mean concentration in (ppb) of trace elements in commercial cow milk sample*.

Brand name	Co	Ni	Cr	Mn	Fe	Zn	Cu	Cd
Nada	4.8	13.4	23.6	9.6	283.7	620.8	28.2	7.8
Al-Marai	5.3	11.2	22.1	22.5	303.8	251.7	27.3	3.1
Al-Rashaka	1.4	8.2	18.3	20.4	299.8	1064.3	43.2	5.1
ABC	3.6	9.3	31.6	13.4	591.7	1080	37	12.2
Juda	7.2	10.1	12.1	17.2	300.1	907.6	41	5.2
Juhaina	8.6	6.5	18.5	18.5	413.6	1200.3	51.6	7.2
Kanoon	5.2	5.2	19.1	21.6	611.4	945.9	23.1	15.6
Yorsan	3.5	10.2	20.2	18.2	287.5	598.6	48.1	4.4
IGium	1	7.3	10.5	17.4	464.2	901.7	22	2
mean	4.51	9.044	19.555	17.644	395.088	841.211	35.722	6.95

**Concentration values are the mean of three aliquots of each brand of fresh cow milk.*

Comparison of the mean concentration of eight elements in human, animal, and commercial cow milk.

A comparison of the mean concentrations of the eight trace elements for the three types of milk samples (human, animal, and commercial cow milk) (Table 5) .It shows that mean Concentrations of Co, Ni, Cr and Cd in commercial cow milk are higher compared to breast and animal milk, whereas the mean concentrations of Mn and Fe in commercial cow milk are in the middle between human and animal milk. While the mean concentrations of Zn and Cu in commercial cow milk are lower than those of human and animal milk.

The distributions of concentration of eight trace elements in (human, animal, and commercial cow milk) samples are shown in Figure 3.

Table 5: A comparison of the mean concentrations of eight trace elements in human milk, animal milk and commercial milk.

Trace elements	human milk	animal milk	Commercial cow milk
Co	2.47	3.375	4.511
Ni	6.56	7.75	10.533
Cr	18.93	13	29.455
Mn	5.04	28	17.64
Fe	564.29	300.5	395.088
Zn	1163.47	1012.5	841.211
Cu	302.15	183	35.722
Cd	6.94	0.73	6.955

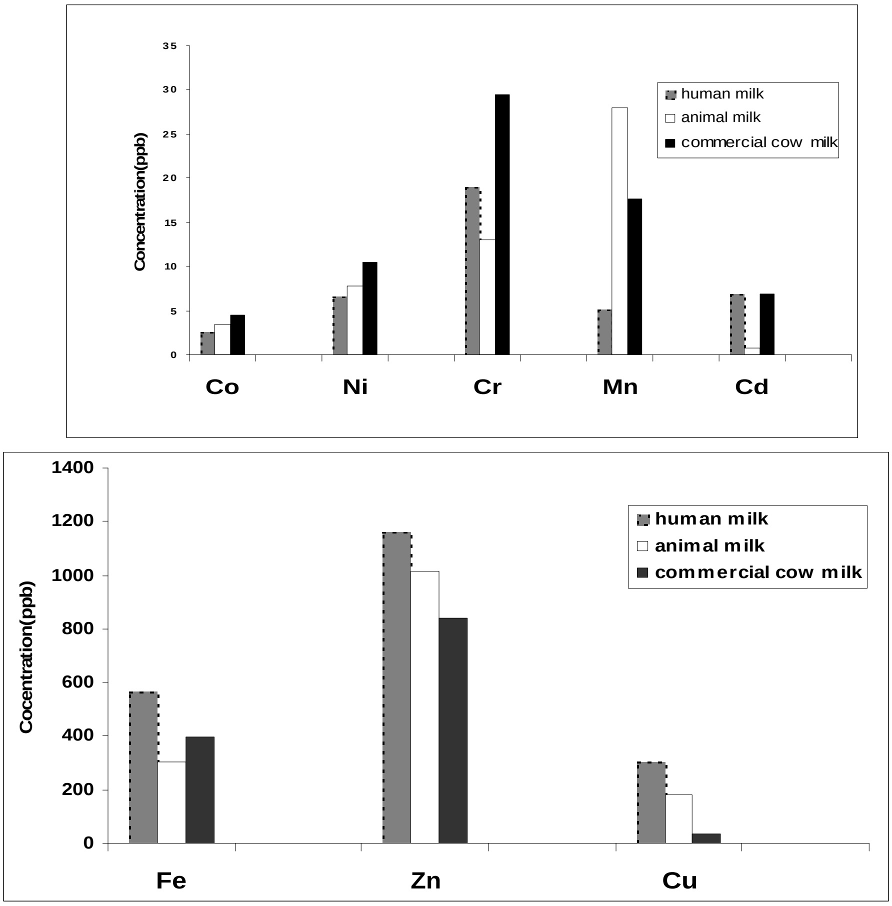

Figure 3: Distribution of concentration of eight trace elements in (human, animal, and commercial cow milk) samples.

Conclusion

The present study determined the levels of the8 trace elements(Mn, Fe, Cu, Zn, Co, Cd, Cr ,and Ni) in milk ,and special attention was given to the comparison of the contents of those trace elements between human , animal (cow, goat) ,and commercial fresh cow milk .It has been reported that contents of several elements in milk could be influenced by some factors, including the lactating period and nutritional status of mothers. Other studies are in progress to elucidate the influence of these factors on the levels of trace elements in milk.

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