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Detection Some Trace Elements in Human Milk and Effect of Some Factors on its Concentrations

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Abstract
Study aim to determination some trace elements in lactating mothers human milk and study effect different factors on its concentration, Age , Body mass index, Home and Smoking habitat, atomic absorption flame less used to measured (Mn, Cd, Co and Zn) in 70 sample of lactating mothers milk which digested using tricolor acetic acid 24%. Result show that concentration of these elements increasing than normal value of these elements,Mn was 1801.40, Cd was 114.64, Co was 288.52 and Zn was 212.10.Also factors were studied effect on minerals concentration, increasing age was non effected on minerals concentration, over weight causes increasing it, urban resident causesincreasing in Mn concentration while rural residence causes increasing in others minerals, negative smoking habitat causes increasing in Cd and Co concentrations.
Key words: trace element, lactating mother's human milk, atomic absorption.

Introduction

Environmental factors increasingly gain importance inpublic health. Children are affected more than adults fromenvironmental deterioration and harmful effects,Children's exposure to environmental hazards may causepermanent damage and will continue during adulthood, Over the past century, there has been an increasing awareness throughout the world of the health and developmental risks associated with environmental exposure to toxic metals, such as, lead (Pb), mercury (Hg), cadmium (Cd), Iron (Fe), Cobalt (Co), and Nickel (Ni). While exposure to toxic levels of any of these environmental contaminants may result in impaired health in adults, the toxicological effects of these metals are often more devastating in the developing central nervous system and general physiological systems of children (landrigan*et al.*, 2002).Environmental pollution in different country orregions, even in rural areas may adversely affect to human life . Air currents, mixing of water sources with metals, the use ofleaded gasoline, lead pipes in drinking water transport, traditional methods of food storage containers in rural sector can make the world more risky in terms of environmentalpollution (Nickerson, 2006).

On the other hand, human milk is also a unique biological matrix for monitoring certainenvironmental contaminants because it can provide exposure information about both themother and the breastfed infant through a non-invasive method of collection. Humanmilk is considered to be one of the most important biota to be monitored for the presenceof Persistent Organic Pollutants (POPs) , Better understanding ofour exposure to harmful environmental chemicals will the help us better manage suchchemicals by eliminating or reducing pollutions emissions of such POPs or by limiting theirpresence in the food supply (EHS. 1998).

There are several chemical forms of minerals in human milk, including inorganic ions and salts, or as constituents of other organic molecules, including proteins, fats, and nucleic acids. They contribute to a variety of physiological functions, including structural components of body tissues to essential parts of many enzymes and biologically important molecules. Sodium, potassium, chloride, calcium, magnesium, phosphorus, and sulfate make up the macro minerals found in human milk. Citrate is found as a water-soluble portion of human milk that can bind some minerals (Tripathi et al., 1999),the primary determinant of macro mineral concentration in human milk is the duration of lactation, during which sodium and chloride decrease, and potassium, calcium, magnesium, and free phosphate increases over time. However, the mineral content of human milk is also influenced by nutritional status of the mother, environmental and other factors (Guo and Hendricks 2007).

Microelements or trace elements are found in human milk in different concentration with different functions and affected by many factors, these microelement include, sodium, potassium, chloride, manganese, and etc… table(2) . Under normal circumstances, a dietary deficiency of sodium, potassium, or chloride does not occur. However, depletion of sodium and chloride can occur during extreme conditions, such as chronic diarrhea, heavy perspiration, or renal disease, and depletion of potassium can occur in situations where there are large alimentary or renal losses (Flynn, 1992).

The concentrations of sodium, potassium, and chloride in breast milk decrease with duration of lactation, from a reported 480, 740, and 850 mg/L in colostrum, respectively, to 160, 530, and 400 mg/L respectively. No relationship has been identified between maternal intake and the concentration of these electrolytes in human

milk. Sodium, potassium, and chloride in human milk are present almost entirely as free ions (Guo and Hendricks 2007).
Cobalt is widely dispersed in the environment humans may be exposed to it by breathing air, drinking water and eating food that contains cobalt. Skin contact with soil or water that contains cobalt may also enhance exposure. Cobalt is not often freely available in the environment, but when cobalt particles are not bound to soil or sediment particles the uptake by plants and animals is higher and accumulation in plants and animals may occur(lanntech, 2012).
Cadmium (Cd), a by-product of zinc production, is one of the most toxic elements to which man can be exposed at work or in the environment. Once absorbed, Cd is efficiently retained in the human body, in which it accumulates throughout life. Cd is primarily toxic to the kidney, especially to the proximal tubular cells, the main site of accumulation. Cd can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. In the industry, excessive exposures to airborne Cd may impair lung function and increase the risk of lung cancer. All these effects have been described in populations with relatively high exposures to Cd in the industrial or in heavily polluted environments.
Manganese (Mn) is a very common compound that can be found everywhere on earth. Manganese is one out of three toxic essential trace elements, which means that it is not only necessary for humans to survive, but it is also toxic when too high concentrations are present in a human body. When people do not live up to the recommended daily allowances their health will decrease. But when the uptake is too high health problems will also occur.(ATSDR, 2000).
The uptake of manganese by humans mainly takes place through food, The foodstuffs that contain the highest concentrations are grains and rice, soya beans, eggs, nuts, olive oil, green beans and oysters. After absorption in the human body manganese will be transported through the blood to the liver, the kidneys, the pancreas and the endocrine glands(lanntech 2012).
Zinc is extraordinarily useful in biological systems. It is involved in many biochemical processes that support life and required for a host of physiological functions, Zinc tends to be corrosive and ingestion can result in severe injury to the mouth, throat and stomach. Initial symptoms include burning of the mouth and pharynx with vomiting and may be accompanied by erosive pharyngitis, esophagitis, and gastritis. Complications
may include gastrointestinal hemorrhage and acute pancreatitis (Prasad, 2008).

Table (1) **Mineral Composition of Mature Human milk (Picciano, 2000).**

Mineral	Concentrations	Mineral	Concentrations
Sodium (mg/L)	207±94	Copper (µg/mL)	0.2-0.4
Potassium (mg/L)	543±78	Manganese (ng/mL)	3-6
Chloride (mg/L)	453±53	Iodine (ng/mL)	12-178
Calcium (mg/L)	259±59	Fluoride (ng/mL)	4-15
Magnesium (mg/L)	31.4±5.9	Selenium (ng/ml)	15-20
Phosphorus (mg/L)	142±25	Aluminum (ng/mL)	4-14
Iron (µg/mL)	0.4-0.76	Chromium (ng/mL)	0.2-0.4
Zinc(µg/mL)	1-3	Molybdenum (ng/mL)	1-2

Martials and methods

Breast milk samples were collected at 1-51 weeks postpartum from the respondent lactating mothers who volunteered to participate in this study.

Before the self manually expressing milk using a conventional breast pump; the breast was washed by distilled waterto a void contamination with environmental menirals.

Ten (10) milliters of milk was collected in sterile polypropylene tube, samples were immediately transferred to a special cooling box with thermometer. The samples were transferred to the chemical laboratory of the DNA

research center lab.In the college of science – Babylon University and stored in at -20C° waiting for analysis for minerals. Data of samples (age, home, habitats, length and weight) were collected in Questionnaire which constructed for present study.
Body Mass Index (BMI) was calculated according to the following equation (Gadzik, 2006) BMI= Weight in Kilograms / Height in square meters

1. BMI between 18.5 – 24.9 = normal
2. BMI between 25- 29.9 = overweight
3. BMI more than 30 = obesity

Milk samples were digested according to Brookes et al., (1970) as follows:
1- Two (1) ml of the human milk were mixed with five (5)ml of the digestive solution (24%TCA W/V) , then shacked well for 30 minutes .
2. The mixture was centrifuged at 4000 rpm for 10 minutes.
3. The supernatant was filtered by filter paper; then the solution was transferred to new tube for detection by the Atomic Absorption spectrophotometer (AAS, Shimadzu .Japan 7000).

Using SPSS version / 17 statistical software, a t-test analysis and the Analysis of Variance (ANOVA - one way) were performed to determine the relationships between different variables. Differences were considered as statistically significant at values $P < 0.05$.

Results

Resultsof present study show that mothers which milk samples collection consist from five categories of age (table 2) under 25 years was more frequented than other, while age above 38 years was low frequented, this because age between 20-37 was perfect age for pregnant in women.
When samples classified according to body mass index, over weight was more frequented than other it was 43.33%, this is because incorrect habitats of feeding during pregnancy and postpartum. Also don't follow daily exercise. Obese was lower percentage of samples it was 23.33%.
According to home 67.18% of lactating mothers were from urban and 31.18% were from rural. No smoking mothers in present study but high percentage of mothers suffer from negative smoking it were 62.85 % while non-smoking mothers were low percentage in present study.

Table (2) Classification of milk samples according to Age, home, BMI and smoking habitat

Categories	**% sample no**	**Categories**	**% sample no**
Age		Home	
20-25	47.69%	urban	67.18%
26-31	35.38%	Rural	31.18%
32-37	9.23%		
38-42	7.69%		
BMI		Smoking habitat	
Normal weight	33.33%	Non smoking	37.14%
Over weight	43.33%	Negative smoking	62.85%
Obese	23.33%		

Trace elements in present study were measured using atomic absorption, four trace elements were measured (Manganese, Cadmium, Cobalt and zinc) in lactating mother's milk in ppm (µg\ml),and then result was analyses according to factors showed in(table 2) to clarify effective of these factors on trace elements concentrations on human milk.
Table (3) Show minerals concentrations according to mothers age categories, Mn was don't effected by age, it increased in third category and decreased in forth. Cd was no affected by increasing age. Cobalt increased in second and forth categories and zinc was trivial changes with age.
Table(3) trace elements concentrations µg\ml in lactating mothers milk according to mother's age

Categories	**Mn**	**Cd**	**Co**	**Zn**
20-25	1837.73±118.29	114.65±0.03	272.34±59.57	212.13±4.63
26-31	1811.65±188.65	114.67±0.05	349.54±87.31	215.54±4.63
32-37	1979.37±254.30	114.53±0.04	138.10±10.17	195.31±6.63
38-42	1518.19±185.99	114.66±0.07	253.52±134.75	210.02±11.57

Me±Se*aova-one way* at ($p<0.05$), no significant
In all studies that deal with evaluation of heavy metals concentrations take body mass index in its consideration for its effected on minerals concentration, in present study Mn was higher in obese but low in overweight, Cd has small changes with BMI, Co increased in overweight and Zn also increased in overweight table (4)Study

suggested that minerals concentrations were increased with body weight but in present study it's don't deal with this role because other factors interacted with BMI such as exposure to heavy metals source, dose of exposure and times of exposure. Also mistakes manner of mothers in their life may be affected on minerals concentrations.Nikniaz*et al* (2011) found significant negative correlation between Fe levels in human milk with BMI when they studied effect different factors on breast milk minerals .Ozkan and others (2007) studied effect lactating period on iron and others milk substances, they found and 4 months after birth .

Iron concentration in the present study decreased with increasing in BMI, this may be because of anemia associated with increasing Pb level, or lactating period (Chierici et al 1999).

Table (4) trace elements concentrations µg\ml in lactating mothers milk according to Body mass index.

Categories	**Mn**	**Cd**	**Co**	**Zn**
Normal weight	1927.14±131.93	114.65±0.06	203.73±6.14	203.97±6.14
Over weight	1538.59±154.08	114.70±0.04	424.68±86.16	220.48±6.14
Obese	2038.70±209.14	114.59±0.04	205.04±79.36	217.63±8.02

Me±Se*anova-oneway* at ($p<0.05$), no significant

Home of lactating mothers affected on milk minerals concentrations in urban Mn was increased while Co and Zn increased in rural. Home affected on minerals concentration resulted from pollution level in urban was higher than rural because present industrials institution and large number of vehicles. Also most mothers in urban are workers and exposure to pollution source was more than others mothers in rural, This may be due to persistent exposure to those environmental pollutants in extensive way in addition to the role of high concentrations of these metals in the working environment or the exposure of women during their transportations to the work place; many local studies explained the high concentrations of these metals in the local environment (air, water and food) (Salman et al., 2007; Hsssan et al., 2010; Taha et al 2005).

Sometimes some minerals are increased in rural than urban because of using some chemical fertilizer which form from minerals, vegetable and water polluted by these fertilizer consider important source of menirals.this may be because this metal can exist in soils in several forms: inorganic crystalline minerals or precipitates, complexed or adsorbed on organic cation surfaces or on inorganic cation exchange surfaces, water-soluble, free-ion or chelated metal complexes in soil solution (Clarkson,1988; Scott-Fordsmand,1997; **.**Bennett ,1982)

Table (5) trace elements concentrations µg\ml in lactating mothers milk according to home

Categories	**Mn**	**Cd**	**Co**	**Zn**
Urban	1976.94±85.16*	114.59±0.28	240.06±47.56	202.57±4.00
Rural	1484.46±172.73	114.73±0.05	428.42±104.91	227.39±9.202

Me±Se*t*-test,* significant at ($p<0.05$),

All study pointed to disadvantage of smoking on human health especially on new born if their lactating mother milk was polluted by minerals. In present study no smoker mothers in samples by negative smoking was higher percentage than no smoking mothers, only Cd and Co were increased in low level. Other factors may be interacted with smoker hesitatealso for detected minerals level and smoker role in human milk study need to further sample of smoker mothers, type of smoking and cigarette number per day.

Smoking habits in the family (smoking during pregnancy and/or being exposed to smoke from other family members)or (negative smoking) increased cadmium concentration in milk .A tendency toward higher cadmium concentration in smokers was observed also in the Swedish and Creation lactating women (Palmiger et al 1995; Frkovic et al ., 1997; Eynon et al ., 1985).estimated that the infants of smoking mothers were exposed 20 - 40 % more to cadmium than the infants from non–smoking mothers and the concentration of cadmium in milk were increased by17 ,18%and 28 % due to the mother's smoking habits before pregnancy; father 's smoking habits and smoking at home, respectively. (Leotsinidis et al., 2005) ,Ursinyova and Masanova (2005) observed higher cadmium concentration in the milk in women from families with smoking habits, but differences were not statistically significant (Ursinyova and Masanova 2005).

Table (5) trace elements concentrations µg\ml in lactating mothers milk according to smoker habitat.

Categories	**Mn**	**Cd**	**Co**	**Zn**
Negative smoking	2162.84±72.33	115.99±1.42	140.53±1.86	191.19±2.51
Non smoking	2261.53±114.07	114.55±1.42	138.68±2.67	194.5±5.26

Me±Se*t*-test at ($p<0.05$), no significant

Total concentration of minerals which detected in present study recorded increased in these concentrations compare with normal value of its in human milk, increasing in minerals concentrations causes different effected on human health especially in new born.

The uptake of manganese by humans mainly takes place through food, such as spinach, tea and herbs. The foodstuffs that contain the highest concentrations are grains and rice, soya beans, eggs, nuts, olive oil, green beans and oysters. After absorption in the human body manganese will be transported through the blood to the liver, the kidneys, the pancreas and the endocrine glands.Chronic Manganese poisoning may result from prolonged inhalation of dust and fume. The central nervous system is the chief site of damage from the disease, which may result in permanent disability. Symptoms include languor, sleepiness, weakness, emotional disturbances, spastic gait, recurring leg cramps, and paralysis. A high incidence of pneumonia and other upper respiratory infections has been found in workers exposed to dust or fume of Manganese compounds. Manganese compounds are experimental equivocal tumorigenic agents(Zhang et al., 2002).

Studies improved that Manganese is a very common compound that can be found everywhere on earth. Manganese is one out of three toxic essential trace elements, which means that it is not only necessary for humans to survive, When people do not live up to the recommended daily allowances their health will decrease. But when the uptake is too high health problems will also occur.high concentrations are present in a human body has been shown to accumulate in maternal liver and to cross the placenta in rats (Jarvinen and Ahstrom ,1975), also increased manganese in the diet of pregnant and lactating rats led to accumulation of manganesein the brains of their offspring, as well as altered concentrations of other metals in their brains (i.e ,.zinc and chromium) (Garcia et al., 2006).

Cobalt is beneficial for humans because it is a part of vitamin B_{12}, which is essential for human health. Cobalt is used to treat anemia with pregnant women, because it stimulates the production of red blood cells. The total daily intake of cobalt is variable and may be as much as 1 mg, but almost all will pass through the body unadsorbed, except that in vitamine $B_{12,}$ too high concentrations of cobalt may damage human health. When we breathe in too high concentrations of cobalt through air we experience lung effects, such as asthma and pneumonia. This mainly occurs with people that work with cobalt. Also plants that grow on contaminated soils they will accumulate very small particles of cobalt, especially in the parts of the plant we eat, such as fruits and seeds. Soils near mining and smelting facilities may contain very high amounts of cobalt, so that the uptake by humans through eating plants can cause health effects. (lenntech,2013).

Recent studies, however, suggest that the chronic low environmental exposure to Cd now prevailing in industrialized countries can adversely affect the kidneys and bones of the general population. These studies show consistent associations between various renal and bone biomarkers and the urinary excretion of Cd used to assess Cd body burden. The public health impact of these findings are still unknown. Further research is needed to ascertain that these associations are truly causal and not secondary to parallel changes in Cd metabolism and in the bone or kidney function occurring because of ageing or diseases unrelated to Cd exposure (Bernard,2008).

Although humans can handle proportionally large concentrations of zinc, too much zinc can still cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anemia. Very high levels of zinc can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to zinc chloride can cause respiratory disorders.(Prasad, 2008).In the work place environment zinc contagion can lead to a flu-like condition known as metal fever. This condition will pass after two days and is caused by over sensitivity. Zinc can be a danger to unborn and newborn children. When their mothers have absorbed large concentrations of zinc the children may be exposed to it through blood or milk of their mothers(Nriagu, 2007).

Table (6) Compatriot between minerals concentrationµg\ml in present study and normal value in human milk.

Minerals	**Mn**	**Cd**	**Co**	**Zn**
Normal value	0.003- 0.006	<0.001	0-0.00044	0.0004-0.008
Present study	1801.40±89.16	114.64±0.027	288.52±43.91	212.10±3.53

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