

Original Research Article

Detection of heavy metals in breast milk and drinking water in Damietta Governorate, Egypt

ABSTRACT

The concentrations of (Manganese, Iron, Zinc, Copper, Lead, Cadmium and Nickel) were determined in 20 samples of breast milk and drinking tap water (10 samples each) collected from Damietta Governorate to comparatively survey these two sources for the accumulation of heavy metals. Heavy metal residues in milk and water were analyzed using the Inductively Coupled Plasma-Mass Spectrometry (ICP-OES). The mean concentrations of Cd, Pb and Zn in milk samples were 1.68 ± 5.36 , 2.92 ± 4.40 and 2.31 ± 5.36 $\mu\text{g/L}$, respectively. While Cu, Fe, Mn and Ni mean concentrations reached to 0.76 ± 0.15 , 2.65 ± 4.16 , 0.25 ± 0.42 and 0.014 ± 0.04 $\mu\text{g/L}$, respectively. The mean concentrations of Cd, Pb, Zn, Cu, Fe, Mn, and Ni in drinking tap water samples were 1.504 ± 1.371 , 1.675 ± 2.426 , 36.692 ± 44.689 , 0.843 ± 1.889 , 4.351 ± 6.052 , 0.196 ± 0.138 and 0.032 ± 0.051 $\mu\text{g/L}$, respectively. This study concluded that the concentrations of Manganese, Iron, Zinc, Copper, Lead and Nickel in drinking tap water and breast milk samples as well as the concentration of Cd in drinking tap water were within the safe limits of WHO regulations. By contrast, the concentration of Cd in breast milk samples was above safe limits and constitutes health hazard to infant.

Key words

Breast milk, heavy metals, drinking water

INTRODUCTION

Milk is considered a perfect natural food for consumers of all age groups due to its high nutritional value; it is high in protein and a valuable source of calcium, vitamins and antioxidants (Zeluta *et al.*, 2009). Breast tissue is unique due to its complex hormonal influences and dramatic changes during various life events. Individual hormonal levels and metabolism are affected by environmental factors, and some frequently used chemical and metals have the ability to disrupt endocrine function, and thus mimic the effects of estrogen (Martin *et al.*, 2006). Metals get into the body through air, food, water, or dermal exposure. Metals have to cross the plasma membrane to enter the cell in order to exert toxicity. If a metal is in a lipophilic form, such as methyl mercury and arsenic compounds, it readily penetrates the membrane (Badawy, 2012). When bound to a protein, such as cadmium metal, the metal is actively taken into the cell by endocytosis (Antila *et al.*, 1996). Human exposure to metals occurred since pre-historic time in areas where the heavy metal content of water and food were naturally high. Other than exist as metal ores in the crust of the earth, metals can also be transported to different parts of the world by various natural cycles. For example, metals, which exist in soil or in ores, can be dissolved by rain. The dissolved metals can then enter river and ground water systems and later the oceans and deposit as sediments. Drinking water is obtained from a variety of sources like wells, tube wells, rivers, lakes, reservoirs and ponds. That poses the greatest risk to human health due to contamination of these sources. Water pollutants mainly consist of heavy metals, microorganisms, fertilizers and thousands of toxic organic compounds. Heavy metals in water occur only in trace levels but are more toxic to the human body (Khan *et al.*, 2011). Metals may be carried up into the atmosphere along with water vapor and subsequently deposit elsewhere. The advancement of technology and industrialization had also contributed to the wider distribution of metals and the increased variety of metal compounds (Hasballah, 2013). Together with essential nutrients, plants and animals also take up small amounts of contaminant heavy metal compounds and can

concentrate them. As certain heavy metals such as lead and cadmium have been recognized to be potentially toxic within specific limiting values, a considerable potential hazard exists for human. Those metals described as "heavy metals" which, in their standard state, have a specific gravity of more than about 5 g/cm³. Some of them, such as copper, nickel, manganese, chromium and iron, for example, are essential in very low concentration for the survival of all forms of life (Watson, 2001). Only when they are presented in greater quantities, can these, like the heavy metals lead, cadmium are already toxic in very low concentrations, causes metabolic anomalies, (Hasballah, 2013). Industry is an important source of heavy metals. Industrial air pollution releases a number of heavy metals into the atmosphere, which become potential sources of water pollution after dry or wet deposition. Agriculture soils are rich in heavy metals because of the use of various phosphate fertilizers, organic matters, and pesticides as well as the presence of decaying plant and animal residue. The use of wastewater in irrigation and sewage sludge has further increased the quantity of heavy metals in agriculture soils. The agricultural run-off together with soil erosion is considered as the potential source of water pollution (Salem *et al.*, 2000). In the last few years, an increase in the number of human diseases in Egypt and in Damietta Governorate specially has been reported. This initiated a multidisciplinary study to disclose the possible role of heavy metals. In Nile branches the water quality deteriorates in a north ward direction due to disposal of municipal and industrial effluent and agricultural drainage as well as decreasing flow (Omran 2016). Damietta branch receives nutrients and high organic loads (Hasballah, 2013). This study seeks to investigate a correlation between the levels of heavy metals (Manganese, Iron, Zinc, Copper, Lead, Cadmium and Nickel) in breast milk and drinking tap water in Damietta Governorate community.

MATERIALS AND METHODS

Sampling

Breast milk and drinking tap water samples were collected randomly from different sites covering four districts in Damietta governorate. Prior to commencing this study, an approval was obtained from The Human Research Ethics Committee at the University of Port Said and The Department of Environmental Sciences at Damietta University to collect and analyze human breast milk. Ten milk samples were collected from adult women (25-40 year) by manual expression into 10 ml acid-washed test tubes. Ten drinking tap water samples were collected from houses into 1000 ml acid-washed polyethylene (PE) bottles. Each breast milk and its corresponding drinking tap water sample were taken from the same house for each region. All samples were transported to the laboratory where they were stored at -4°C until analysis at the Central Laboratory, Accurate Analysis Unit, Tanta University. Accurate Analysis Unit.

Analysis of heavy metals

I. In human breast milk

The concentration of heavy metals in collected milk samples was estimated according to the method of (AOAC, 2000a). Briefly, 1 ml aliquot of each milk sample was placed into a clean silica dish and allowed to dry on a hot plate. When the samples were thoroughly dry, the contents of the dish were further dried with a soft flame (such as that of a Bunsen burner) until all volatile or readily combustible matter has been removed. The plates were then transfer to a furnace set at 250 °C and the temperature was gradually raised to 550 °C for about 6 to 8 hours. The dishes were removed and allowed to cool to room temperature. At this point, ashes were expected to be white or brownish red and essentially be carbon free. Ashes were mixed with 1ml of HNO₃ (Redistilled, Sp. Grade 1.42) and 10 ml of water and

heated on hot plate until ashes have been dissolved. Dissolved ashes were cooled down before gently adding 1 to 2 ml of nitric acid and water to dissolve the salts. Dissolved samples were filtered into 25 ml volumetric flask using Whatman No. 44 filter paper. The residue and basin were washed twice by water and volume was made up Make up to volume with water. Prepare sample blank solution by following the same procedure as described for sample. Use same quantities of reagents including water for both sample and blank. Subject both sample and sample blank to identical treatment (even the length of time kept in furnace etc.).

II. In drinking water sample

Heavy metals concentration in drinking water was determined according to method of (AOAC, 2000b). A volume of 100 ml of sample was measured into a beaker and 5 ml concentrated HNO_3 were added. The mixture was boiled slowly on a hot plate or a steam bath until the volume has evaporated down to about 20 ml. A further 5 mL of concentrated HNO_3 were added and the beaker covered with a watch glass and heated. Heating and adding 4concentrated HNO_3 were continued until the solution has appeared light colored and clear. A 1-2 ml aliquot of concentrated HNO_3 was added and the mixture heated slightly to dissolve any remaining residues. The beaker walls and the watch glass were washed down with water which then is transferred to a 50 mL volumetric flask, cooled and the volume made up to the mark with water. The Inductively Coupled Plasma-Mass Spectrometry (ICP-OES 7000) was used for heavy metal analysis with ultrasonic nebulizer (USN), this Nebulizer decrease the instrumental detection limits by 10%, this ICP instrument is Perkin Elmer Optima 3000, USA

RESULTS AND DISCUSSION

Heavy metals have the same pathway of exposure, emissions from industry and traffic exhaust contains considerable air contaminants, which reach the food chain and then animals to finally deposit in human body (El-Zeiny, 2010). In human breast milk, concentrations of heavy metals followed the order $\text{Pb} > \text{Fe} > \text{Zn} > \text{Cd} > \text{Mn} > \text{Cu} > \text{Ni}$ (Table 1). The concentrations of Pb and Fe were high in all the sampling regions with mean concentrations of 2.921 ± 4.406 and 2.655 ± 4.166 $\mu\text{g/l}$ breast milk, while Ni with mean concentration of 0.014 ± 0.041 $\mu\text{g/l}$ recorded the lowest concentration. The toxic metal Pb achieved high concentration, which considered high risk for humans.

The concentrations of heavy metals in drinking tap water samples, as shown in Table 2, followed the order $\text{Zn} > \text{Fe} > \text{Pb} > \text{Cd} > \text{Cu} > \text{Mn} > \text{Ni}$. Zinc and iron had high concentrations in all the sampling regions with mean concentrations of 36.692 ± 44.689 and 4.351 ± 6.052 $\mu\text{g/l}$, respectively. In contrast, a lower value was observed for Ni with mean concentration of 0.032 ± 0.051 $\mu\text{g/l}$ milk sample. As shown in Figure 3, breast milk samples had higher concentrations of Cd, Pb and Mn than drinking tap water samples, whereas concentrations of Zn, Cu, Fe and Ni in drinking water samples were higher in drinking water than in breast milk samples. Damietta governorate, by its four districts, is considered a commercial, industrial and agricultural area that is rapidly growing and suffers severe anthropogenic pressure due to 1) the presence of petrochemical plants, 2) the widespread of several industrial activities including fertilizers, pesticides, plastics and chemicals manufacturing, dyeing and printing and 3) many workplace parts such as, construction materials, furniture, car service and others, and 4) to consequent high traffic density. Accumulation of heavy metals, including Pb, Zn, Mn, Cd and Cu, in a certain area arise from vehicle exhausts, industrial discharges, oil lubricants, automobile parts, corrosion of building materials, and atmospheric deposition. Heavy metals can enter a water supply by industrial and consumer waste, or even from rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater (El-Zeiny, 2010). High levels of zinc through study samples and regions may be contributed to increasing use of zinc substances, which contact with

galvanized copper or plastic pipes (**Bakircioglu et al., 2011**). In addition, the discharges of industrial wastes such as a municipal solid waste (MSW) compost could result in increased Cu, Pb and Zn in soil (**Carbonell et al., 2011**). In commercial areas with high population and traffic sources, where zinc in accumulators of motor vehicles or in carburetors, zinc may come from lubricating oils and tires of motor vehicles, in addition to organo-zinc compounds that are used as fungicides, topical antibiotics (**Arslan, 2001**) and the basic metal pesticides (**Watson, 2001**). Zinc released to water (**US. EPA, 1980**) then to soil for irrigation reaching to crops then to animal which consumed contaminated plants with Zn. High concentration of iron can be natural whereas the increased concentrations of other heavy metals were due to soil pollution, vehicular and industrial emissions and crust re-suspension. Iron is originated mainly from the soil type and construction work materials. It is also clear that auto-exhaust Fe particulates were added to the natural Fe, which is a major constituent of earth crust (**El-Zeiny, 2010**). This is in agreement with **Habibi (1973)** who found that the major source of Fe in the city centre was the auto-exhaust where Fe constituents ranged from 30 to 53 % of emitted particles. **Balachandran et al. (2000)** had stated also that the high concentration of Fe can be attributed to the open and rocky terrain of the area which is more susceptible to the condition of re-suspension of soil dust. Finally, heavy metals are extremely persistent in the environment; they are non-biodegradable and non-thermo degradable and thus readily accumulate to toxic levels. Heavy metals can accumulate in the soil at toxic levels due to the long-term application of wastewater. The primary natural source of nickel in drinking water is leaching from ultramafic rocks and the soil derived from these rocks. It is found primarily combined with oxygen (oxides) or sulfide Nickel compounds that are also used in for Nickel plating, to color ceramics, to make some batteries and as catalyst in different chemical processes. These may be the possible means for water contamination. Nickel is required to maintain health in animals. A small amount of nickel may be essential for human, although a lack of nickel has not been found to effect peoples' health (**Khan, 2011**). One important dietary uptake pathway could be through crops irrigated with contaminated wastewater. Soils irrigated by wastewater accumulate heavy metals such as Cd, Zn, Cr, Ni, Pb, and Mn in surface soil. When the capacity of the soil to retain heavy metals is reduced due to repeated use of wastewater, soil can release heavy metals into ground water or soil solution available for plant uptake (**Sharma et al., 2006**). According to the data in Table (3), the concentrations of Pb, Ni, Zn, Mn, Cu and Fe in drinking tap water and breast milk samples as well as the concentration of Cd in drinking tap water were within the safe limits of WHO regulations (**WHO, 1993, 2003**). By contrast, the concentration of Cd in breast milk samples was above safe limits (**WHO, 1993**). The results agree with (**Ghanem et al., 2007**) who found high concentrations of Pb in mothers milk and was above the permissible value established by WHO.

CONCLUSION

This comparative study revealed that the concentrations of determined heavy metals in drinking water samples collected from Damietta Governorate were below the international permissible limits (**WHO, 1993, 2003**), while only the mean concentration of Cd in the breast milk samples was higher than international permissible limits (**WHO, 1993**). Cd contaminations in the breast milk samples have potential for human health risk. Therefore, it is recommended that drinking tap water should be regularly monitored for heavy metals in order to prevent excessive buildup of these heavy metals in the human food chain.

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Table (1): Concentration of heavy metals in mother breast milk in different sites from Damietta.

Sites	Heavy Metals ($\mu\text{g/l}$)						
	Pb	Zn	Mn	Cu	Fe	Ni	Cd
1	4.889	17.27	0.243	0.401	1.141	0.001	4.6
2	14.93	3.268	1.449	0.353	14.04	0.132	10.17
3	1.584	0.003	0.048	0.001	0.763	0.001	0.668
4	0.655	0.003	0.163	0.001	0.973	0.001	0.001
5	0.845	0.003	0.048	0.001	0.364	0.001	0.263
6	0.32	0.855	0.132	0.001	4.38	0.001	0.67
7	2.045	1.697	0.153	0.001	2.241	0.001	0.04
8	1.226	0.003	0.109	0.001	1.032	0.001	0.02
9	1.105	0.003	0.136	0.001	0.779	0.001	0.177
10	1.612	0.003	0.055	0.001	0.84	0.001	0.218
*Mean $\pm$ SE	2.921 $\pm$ 4.40	2.311 $\pm$ 5.36	0.254 $\pm$ 0.42	0.076 $\pm$ 0.15	2.655 $\pm$ 4.16	0.014 $\pm$ 0.04	1.683 $\pm$ 5.36

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Table (2): Concentration of heavy metals in drinking tap water in different sites from Damietta

Sites	Concentration of Heavy Metals ($\mu\text{g/l}$)						
	Pb	Zn	Mn	Cu	Fe	Ni	Cd
1	8.333	32.35	0.174	1.227	1.469	0.103	4.022
2	0.8	0.003	0.098	0.21	1.726	0.001	2.298
3	0.8	0.003	0.098	0.21	1.726	0.001	2.298
4	0.544	107.5	0.353	0.333	15.76	0.107	2.445
5	0.544	107.5	0.353	0.333	15.76	0.107	2.445
6	0.495	46.67	0.003	6.117	1.343	0.001	0.049
7	1.672	0.048	0.136	0.001	0.699	0.001	0.115
8	2.18	71.4	0.286	0.001	0.206	0.001	0.414
9	0.003	0.003	0.073	0.001	2.241	0.001	0.801
10	1.378	1.44	0.389	0.001	2.576	0.001	0.156
*Mean $\pm$ SE	1.675 $\pm$ 2.426	36.692 $\pm$ 44.689	0.196 $\pm$ 0.138	0.843 $\pm$ 1.889	4.351 $\pm$ 6.052	0.032 $\pm$ 0.051	1.504 $\pm$ 1.371

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Table (3). Permissible Limits for heavy metals of drinking water and breast milk

Permissible Limits	Metals (mg/l)						
	Cd	Cu	Ni	Mn	Pb	Fe	Zn
(WHO, 1993) in drinking water	0.003	-	-	0.5	0.01	-	3
(WHO, 2003) in drinking water	0.003	-	0.02	-	-	0.3	3
(WHO, 1993) in breast milk	0.0001			0.018	0.005		

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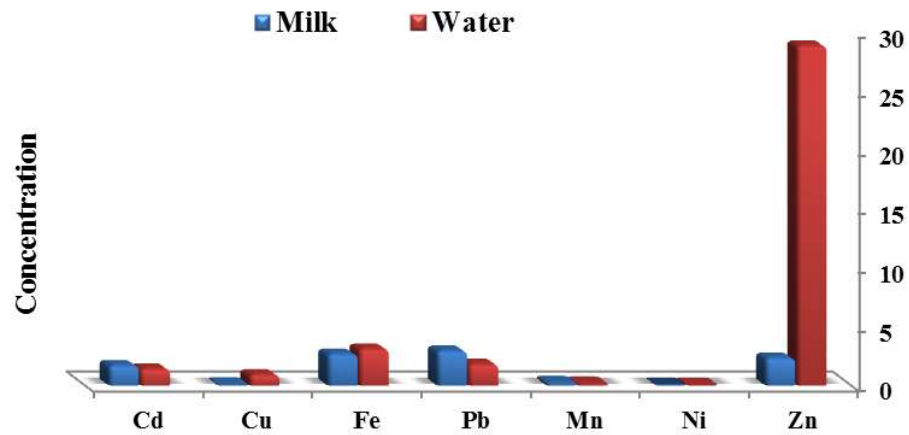


Figure 3: Comparison between heavy metal mean concentrations in drinking tap water and breast milk samples.