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ASSESSMENT OF HEAVY METALS POLLUTION IN GROUNDWATER AND COW'S MILK IN UPPER EGYPT

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ABSTRACT

The present work deals with the evaluation of heavy metals pollution in raw cow's milk and groundwater in Upper Egypt. So, a total of 102 milk and ground water samples were collected from three Governorates in Upper Egypt, namely, Assiut, Sohag and Qena. Concentrations of lead (Pb), Cadmium (Cd), Iron (Fe) and Manganese (Mn) in the digested water and milk samples were determined by Buck model 210 VGP atomic absorption spectrophotometer with flame atomization. Our data showed that the average mean of lead, Iron and cadmium in collected water and milk samples were (0.195 ppm and 0.075 ppm), (0.00040 ppm and 0.00026 ppm) and (3.949 ppm and 0.869ppm), respectively. However, we could not detect Manganese either in milk or water samples. Furthermore, Statistical analysis of data showed that there was a significant correlation ($p < 0.05$) between heavy metal pollution in milk and that of correspondent examined groundwater samples.

Key words: *Heavy metals, cow, milk, groundwater, pollution.*

INTRODUCTION

The environmental pollution with heavy metals is an issue of global dignified concern and wherefore pollution of food chain is fetching further significance in view of its detrimental aspect in human health and nutrition (Pilarczyk *et al.*, 2013 and Jalilian and Saber, 2015). Heavy metals pollution is an ecumenical hazard to the environment as they are widely distributed in the earth's crust, air, water and food chain (Matthew *et al.*, 2002 and Szkoda *et al.*, 2013B).

There is no doubt that water may act as a source of infection and toxicity among domestic animals. Drinking of polluted water by toxic heavy metals have been responsible for health problems in dairy farms and human beings (Rajaganapathy *et al.*, 2011 and Mohod and Dhote, 2013). However, water used in the dairy farms either for drinking of livestock, washing of udder and utensils as well as that used for washing of the stable must be free from the toxic heavy metals.

An adequate supply of clean, fresh drinking water is widely considered essential for optimal cow health and maximum milk production (Radostitis *et al.*, 2007; Beede, 2009 and Awasthi *et al.*, 2012). The increment of heavy metals pollution in the ecosystem may be attributed to use of agricultural pesticides and related chemicals, unhygienic weed out wastes and diffusion of sewage quagmire (López-Alonso *et al.*, 2003; Srikanth *et al.*, 2004; Miranda *et al.*, 2007; Chiroma *et al.*, 2007 and Tona *et al.*, 2013).

Milk is an essential diet for children as well as for adults due to its protein and mineral contents, contamination of these products is a major public health problem in many countries (Inam and Somer, 2000; Pilarczyk *et al.*, 2013). Milk polluted with heavy metals is considered a vehicle for transmission of illness among consumers, especially in developing countries where food hygiene is still under-way due to lack of food hygienists, modern diagnostic methods, beside some environmental conditions and financial difficulties (EL atrash and Atoweir, 2014).

If lactating cows are exposed to high quantities of toxic metals, such as cadmium and lead, these metals disturb different metabolic activities as well as health of children (González-Weller *et al.*, 2006; Vromman *et al.*, 2008 and Cai *et al.*, 2009). Enormous exposure of heavy metals such as cadmium, lead, iron and mercury is pernicious for plants, animals and human beings (Llobet *et al.*, 2003; Malhat *et al.*, 2012 and Mohod and Dhote, 2013).

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Estimation of the residual concentrations of heavy metals in milk could be a significant indicator of the hygienic status of the milk, as well as the degree of pollution of the environment in which the milk was produced (Licata *et al.,* 2004 and Gonzalez-Montana *et al.,* 2012).

Due to growing heavy metals pollution threatening human and animal health, therefore the main goal of this study was to assess the status of drinking water quality in Upper Egypt and its impact on cow's milk pollution.

MATERIALS and METHODS

1. Source of specimens:

1.1. Animal housing:
This study was conducted in 51 dairy cow's houses during the period from January to June, 2015. Each animal house associated with owner's houses in 3 different villages that equally distributed in three Governorates in Upper Egypt, namely Assiut, Sohag and Qena.

2. Sampling:
A total of 102 samples were taken as 51 milk and 51 water samples (34 samples from each Governorate). Each sample was taken under strict hygienic conditions and labeled to indicate type, date, time…etc, and then carried with a minimum of delay for chemical examination.

2.1. Water samples:
Fifty-one water samples were collected from the examined animal houses. The source of samples was groundwater in the three Governorates (one sample from each house). Each water sample was collected in clean dry plastic bottles previously rinsed by nitric acid fitted tightly with ground glass stopper (according to the recommendation of APHA, 2005). Before collection of samples, thoroughly cleaning of the tap nozzles of driven pumps was done, then water was run for 3-4 min to rinse any accumulated dust and dirt.

2.2. Milk samples:
Firstly, the teat apices were cleaned, then the first stream of quarter was discarded, and about 20 ml of milk was drawn from each quarter into a sterile 250 ml capacity glass bottle. Milk sampling was done according to the recommendation of National Mastitis Council (1999).

3. Preparations of samples:
3.1. Glasswares were washed before use with distilled water, soaked in nitric acid (30%), then rinsed in distilled water and air dried. All digestion tubes were identified for examination.

3.2. Water samples were digested by using a mixture of concentrated nitric and perchloric acids according to (Chau *et al.,* 1979). Five milliliter of each sample was transferred to a digestion flask where it was treated with 5 ml of nitric and perchloric acids mixture (HNO3: HCLO4 = 4:1 v/v). The samples were left to be stand for the cold digestion overnight, and then were heated on a hot plate (model 1030-RuMO 100) at 70˚C till disappearance of the brown fumes of NO3 and the sample become clear. After cooling, each sample was diluted to 25 ml with bi-distilled water and filtered through ashless filter paper (Whatman paper). The digested samples were kept refrigerated in 50 ml propylene bottles till analysis.

3.3. Milk samples
Milk samples were processed for wet digestion where 1 ml of each sample was digested by 5 ml of concentrated nitric acid (HNO3) (Riedel-de Haen®) and 5 ml of concentrated perchloric acid (HCIO4). The samples were left to be stand for the cold digestion overnight, and then were heated on a hot plate (model 1030-RuMO 100) at 70˚C till disappearance of the brown fumes of NO**3** and the sample become clear. After cooling, each sample was diluted to 25 ml with bi-distilled water and filtered through ashless filter paper (Whatman paper). The digested samples were quantitatively transferred into 50 ml flask, made up to the mark with distilled water and stored in 50 ml propylene bottles as described by Debeca and Mckenzie, 1992 and Tsoumbaris and Tsoukali-Papadopulou (1994).

4. Measurement of heavy metals in milk and water samples:
Concentrations of lead (Pb), Cadmium (Cd), Manganese (Mn) and Iron (Fe) in the digested water and milk samples were determined by Buck model 210 VGP atomic absorption spectrophotometer with flame atomization. Buck Scientific Inc. East Norwalk, CT (USA), in the lab of Faculty of Science, Assiut University, Egypt.

3. Statistical analysis
Analysis of variance of data was computed using the General Linear Models (GLM) Procedure of SAS software version 9 (SAS, 2009). The mean of heavy metals concentrations and standard error were measured in milk and water and comparison between the milk and water was done using t-test. Pearson Correlation was made to measure the correlation between the estimated variables. P-value was considered statistically significant when $p<0.05$.

RESULTS

Table 1: Heavy Metals levels in the examined cow's milk (ppm)

Metal	Governorates	Milk			Maximum Permissible Limit (ppm)
		Minimum	Maximum	Mean ± SE	
Lead (Pb)	Assiut	0.013	0.143	0.067 ± 0.043	**0.02** **according to Codex Alimentarius commission (2007) and According to IDF Standard (1979)**
	Sohag	0.001	0.220	0.084 ± 0.055	
	Qena	0.02	0.134	0.073 ± 0.035	
	Total houses	0.02	0.220	**0.075 ± 0.044**	
Cadmium (Cd)	Assiut	0.0001	0.0006	0.00027 ± 0.0001	**0.0026** **According to IDF Standard (1979)**
	Sohag	0.00010	0.003	0.00031±.0.0008	
	Qena	0.00011	0.003	0.00035± 0.0009	
	Total houses	0.00010	0.003	0.00026± 0.00011	
Iron (Fe)	Assiut	0.508	1.31	0.840 ± 0.247	**0.037** **According to IDF Standard (1979)**
	Sohag	0.571	1.374	0.911 ± 0.321	
	Qena	0.522	1.103	0.855 ± 0.238	
	Total houses	0.571	1.374	**0.869 ± 0.265**	
Manganese (Mn)	Assiut	0	0	0.000 ± 0.000	
	Sohag	0	0	0.000 ± 0.000	0.05 **According to Wenlock *et al.* (1979)**
	Qena	0	0	0.000 ± 0.000	
	Total houses	0	0	**0.000 ± 0.000**	

Table 2: Heavy Metals levels in the examined groundwater samples (ppm)

Examined Metal	Governorates	Groundwater			Maximum Permissible Limit according to WHO, 2011 (ppm)
		Minimum	Maximum	Mean ± SE	
Lead (Pb)	Assiut	0.144	0.261	0.187 ± 0.036	**0.01**
	Sohag	0-137	0.324	0.230 ± 0.068	
	Qena	0.087	0.341	0.168 ± 0.083	
	Total houses	0.144	0.341	**0.195 ± 0.07**	
Cadmium (Cd)	Assiut	0.0001	0.0006	0.00031± 0.00017	0.003
	Sohag	0.0003	0.0006	0.00042± 0.00015	
	Qena	0.0003	0.0008	0.00049± 0.00026	
	Total houses	0.0001	0.0008	0.00040± 0.00021	
Iron (Fe)	Assiut	1.833	5.052	3.456 ± 1.162	0.3
	Sohag	2.457	6.301	4.417 ± 1.244	
	Qena	2.08	6.333	3.975 ± 1.725	
	Total Farms	2.457	6.333	**3.949 ± 1.416**	
Manganese (Mn)	Assiut	0	0	0.000 ± 0.000	0.1
	Sohag	0	0	0.000 ± 0.000	
	Qena	0	0	0.000 ± 0.000	
	Total houses	0	0	**0.000 ± 0.000**	

Table 3: Statistical analysis of heavy metal in milk and water samples

Metal	Examined samples	
	Milk	water
Lead	0.075 ± 0.044*	0.195 ± 0.07*
Cadmium	0.00026 ± 0.00011*	0.00040 ± 0.00021*
Iron	0.869 ± 0.265*	3.949 ± 1.416*
Manganese	Not detected	Not detected

*: Means with superscripts are significantly related at P< 0.05.

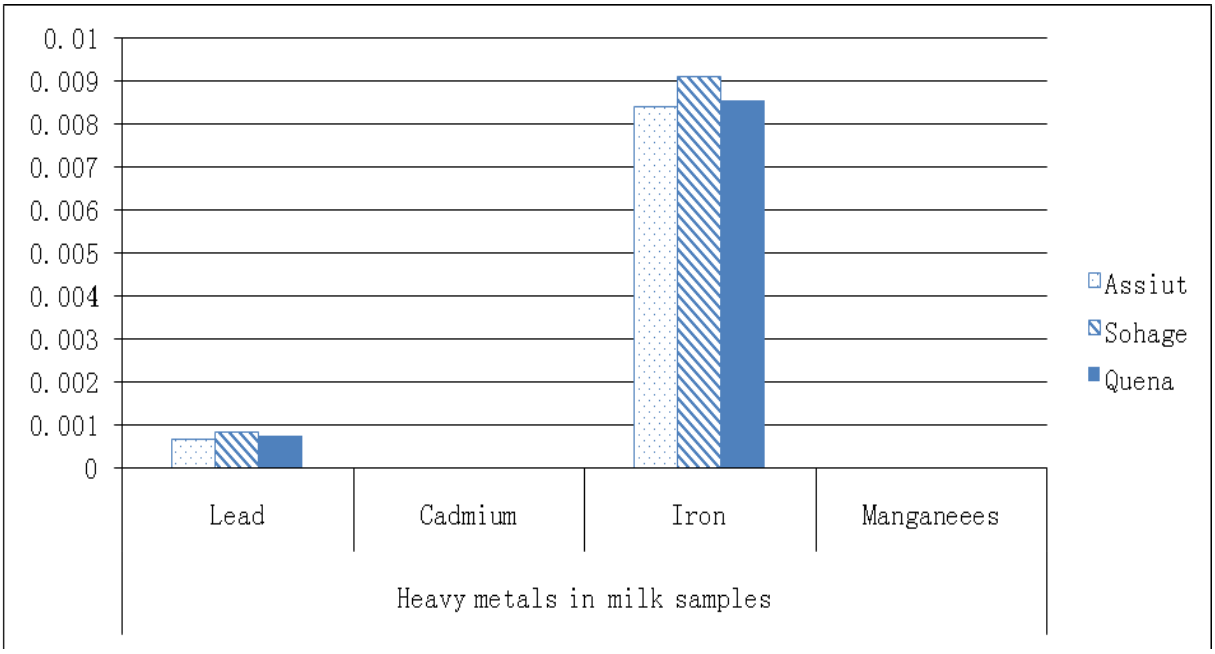

Fig. 1: Diagram of heavy metals concentration in cow's milk samples of the different three governorates.

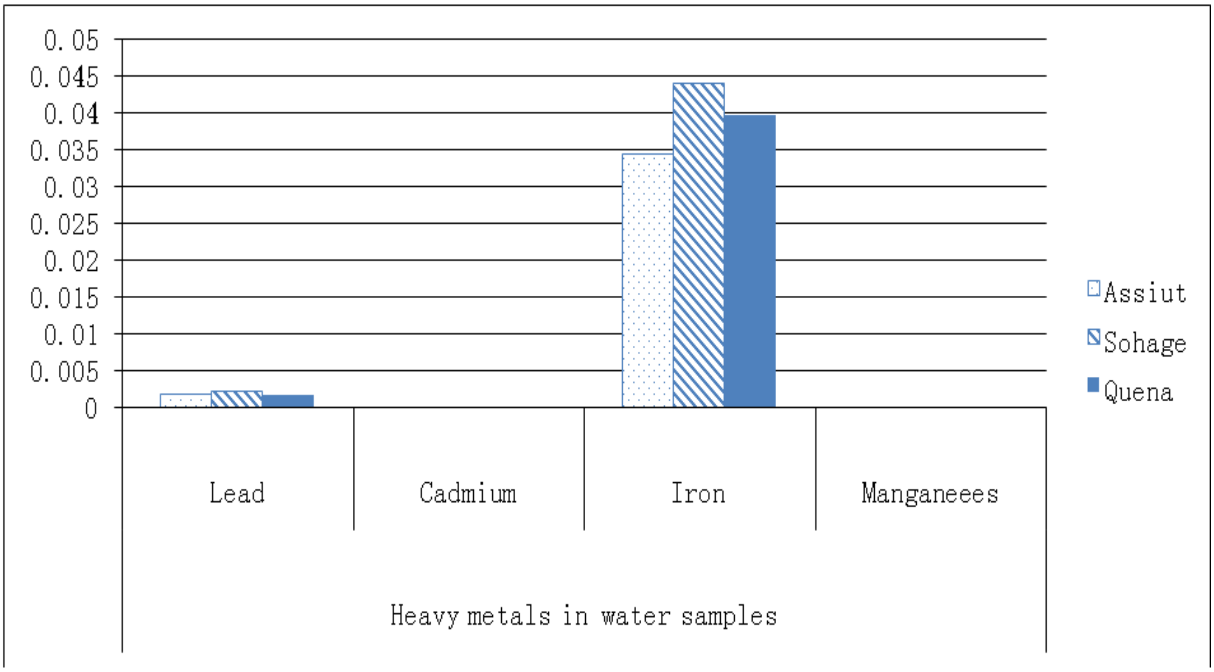

Fig. 2: Diagram of heavy metals concentration in ground water samples of the different three Governorates

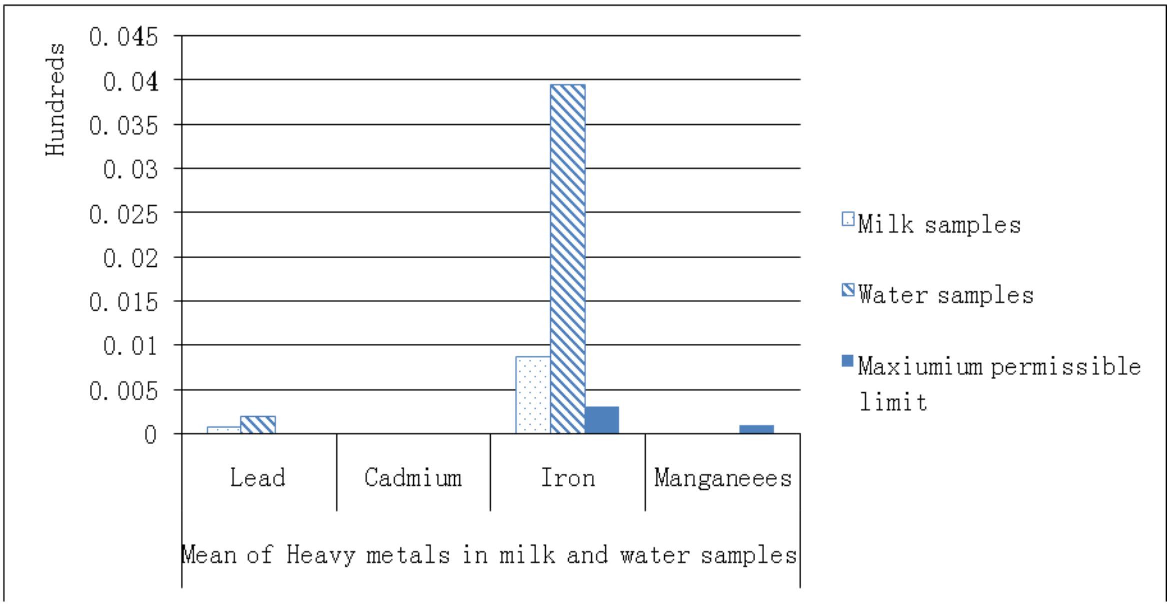

Fig. 3: Diagram of Mean heavy metals concentration in ground water and cow's milk samples

DISCUSSION

1. Heavy metals in milk

Excretion of milk from bovine mammary glands can convoy abundant of xenobiotic elements, which constitute a health hazard risk for the consumer. Measurement of the residual concentrations of metals in milk could be one of the most important steps in determining the hygienic status of the milk and the extent of environment pollution in which the milk was produced (Licata *et al.*, 2004; González-Montaña *et al.*, 2012 and Pilarczyk *et al.*, 2013).

The concentration of different examined heavy metals in milk samples is given in table 1 and figure 1.

The results of table (1) and figure (1) showed that there was variation between the mean level of Lead concentration of milk samples at different Governorates, where the highest concentration found in Sohag Governorate (0.084 ppm) followed by Qena (0.073 ppm) and Assiut (0.067 ppm), respectively. The same table showed that the mean concentration of lead of all examined milk samples from all different houses was 0.075 ± 0.044 ppm. This finding more or less in agreement with the results of Pavlovic *et al.* (2004); Gabryszuk *et al.* (2010); Bilandžić *et al.* (2011) and Malhat *et al.* (2012).

Concerning Cadmium concentration in milk samples, we found that the mean of cadmium contents in the examined milk samples of Assiut, Sohag and Qena Governorates were 0.00027 ppm, 0.00031 ppm and 0.00035 ppm, respectively. However, the mean content of cadmium in all milk samples was 0.00026 ppm.

This result was less than that detected with Malhat *et al.* (2012), who found that Cd ranged from 0.200 to 0.288 ppm in milk samples.

In regarding to Iron, from our study, we noticed that the mean content of lead in examined milk samples was 0.869 ppm. This finding was lower than that detected by Malhat *et al.* (2012) who found that the mean Fe concentration in the milk samples varied from 10.95 to16.38 ppm. Also, our result was lower than Haldar *et al.* (2003) and Santos *et al.* (2015) who reported Fe in cow's milk varied from 3.97 to 4.19 ppm.

Concerning Manganese concentration in milk samples, we failed to detect it in all examined milk samples from different examined animal houses. This result was coincided with Lante *et al.* (2006). On the other hand, our result was disagreed with Haldar *et al.* (2003) and Santos *et al.* (2015) who found Mn in cow's milk with the range of 1.60 to 1.69 ppm.

The maximum permissible levels of heavy metals recommended by international dairy federation standard (IDF Standard 1979) are 0.037 ppm for Fe, 0.02 ppm for Pb, and 0.0026 ppm for Cd. According to Codex Alimentarius Commission (2007) the maximum residues limit for Pb in milk is 0.02 ppm.

The average concentration of the Lead and Iron in milk samples was compared with the maximum permissible limits. Our results showed that all milk samples collected from the different Governorates containing Lead and Iron with concentration higher than those recommended for milk by IDF standard, 1979. Lead is an environmental pollutant which is toxic to humans and animals (Cai *et al.,* 2009). As well as Pb is non-biodegradable, and their accumulation in the environment raises agricultural and public health concerns (Olsson *et al.,* 2005 and De Vries *et al.,* 2007). Unfortunately, the lead content in the milk of cows was higher than the permissible concentration of 0.02 ppm in the raw. The higher content of Pb in milk may be attributed to industrial air pollution in this area especially all houses located at a traffic ways, the use of contaminated feed, and mineral supplements as well as water supply of bad hygienic quality (Antunovic *et al.,* 2005; Rodrigue *et al.,* 2009; Abdulkhaliq *et al.,* 2012 and Santos *et al.,* 2015).

Iron play a crucial role in protecting the body against the negative effects of toxic free radicals (Santos *et al.,* 2015). As well as iron promotes development of oxidized flavor in milk and milk products. They also catalyze oxidation of ascorbic acid and influence lipase activity (Mondal *et al.,* 2015). However, Fe can represent a problem in dairy technology because of its catalytic effect on oxidation of lipids with development of unpleasant smell, bounding preferably proteins and membrane lipoproteins of milk fatty globule (Lante *et al.,* 2006). Iron overdoses manifestation in human is dehydration, hypovolemia, evidence of hepatic necrosis (elevated alanine transaminase and aspartate transaminase activities) and liver failure (Greentree and Hall, 1995 and Hillman, 1995).

2- Heavy metals in water

Water is the most important nutrient for lactating dairy cattle. It is required in a larger quantity than all other nutrients combined. Without an adequate clean, fresh supply of water every day, milk production will be severely compromised.

From the mentioned data (table 2 and figure 2), we noticed that the average mean of Lead content in water samples was 0.195 ppm with the highest and lowest mean contents were found in water samples collected from Sohag houses (0.230 ppm) and Qena houses (0.168 ppm), respectively.

All the examined water samples contained lead concentration above the levels permitted by WHO (2011). The main sources of lead contamination are industrial discharges from smelters, battery manufacturing units, run off from contaminated land areas, atmospheric fall out and sewage effluents. The levels of Pb from this study were lower than study reported by Nassef *et al.* (2006) and Wongsasuluk *et al.* (2014) and higher than those reported by Boateng *et al.* (2015).

Source of lead in drinking water is mainly through the corrosion of lead-bearing pipe materials which located adjacent to sewage effluent and water canals and drains used to irrigate agricultural land as in our examined houses (Javed *et al.,* 2009; Brown *et al.,* 2011; Triantafyllidou and Edwards, 2012; Edwards, 2014 and Masters and Edwards, 2015).

Maximum allowable level of Lead in drinking water is 0.01 ppm according to WHO (2011). But today no level of lead is considered to be safe (Dettwyler, 2000) as it produces a strong negative effect on human and animal health (Bhat and Moy, 1997). Today everyone is exposed to environmental lead in the form of industrial wastes, leaded gasoline and other anthropogenic sources (Khillare *et al.,* 2004 and Al-Masri *et al.,* 2006). Air pollution is very common in the big cities because of the vehicles burn gasoline containing lead. It is thought that lead is responsible for number of deaths (Oroian *et al.,* 2007).

The obtained data given intable (2) and figure (2) showed that the cadmium contents in Assiut, Sohag and Qena governorates were 0.00031 ppm, 0.00042 ppm and 0.00049 ppm, respectivily with overall mean content of 0.00040 ppm and this result was not coincided with Boateng *et al.* (2015) and Gimba *et al.* (2015).

Cadmium concentrations in palatable water are up to 0.01 ppm considered as permissible limit (WHO, 2011). Groundwater barely contains huge amount of cadmium except if it is polluted by industrial wastewater, tailings from mining or leakage from precarious waste sites (ATSDR, 2008).

It is fortunate that the content of Cadmium in milk and water samples in all governorates was under maximum permissible limit. However, further studies should be conducted to assess heavy metal concentration in animal tissues and organs as well as other environmental samples as soil and air. It has been found that cadmium has not a single physiological function within the human body. Therefore, attention has been diverted to its bio-hazardous potential. Once cadmium is absorbed, it accumulates in the body even throughout the life (Bernard, 2008). Even low concentration of cadmium can adversely affect the number of metabolic processes in animal body (Bernard, 2004 and Nordberg *et al.,* 2007). Cadmium intoxication can lead to kidney, bone and pulmonary damages (Godt *et al.,* 2006). Cadmium toxicity affects various organs such as the liver, lung, testis and hematopoietic system in animals (Kocak and Akc, 2006).

An acute toxicity symptom generally includes abdominal and muscular cramps, headache, overtiredness, shock and ultimately death (USAF, 1990). Cadmium is also absorbed in significant quantities from cigarette smokes which ultimately cause toxic effects on both human and animal health. The deleterious effects are especially on kidneys, liver and vascular system but most undesirable effects have been seen on reproductive tissues and developing embryos (Thompson and Bannigan, 2008).

Also, we found from the data present in table (2) and figure (2) that the iron content in water samples ranged from 1.833 ppm to 6.33 ppm with the mean value of 3.949 ppm in all examined water samples. Our result was more or less coincided with data reported by Ansa-Asare *et al.* (2009); Boateng *et al.* (2015), Gimba *et al.* (2015) and Masters and Edwards (2015).

The maximum permissible limit reported by WHO (2011) is 0.3 ppm for iron, all examined water samples were observed to be above the maximum permissible limit set by the world health organization(WHO) for drinking water. This indicates that the local mineral deposit in the studied area may have high levels of iron.

Chronic consumption of water with iron overload may results in fatigue, weight loss, joint pains and ultimately heart disease, liver problems and diabetes (US-CDC 2011).

Contrastingly**,** the obtained data given in table (2) and figure (2) showed that there is no manganese in all examined water samples and this result was not coincided with Boateng *et al.* (2015) and Gimba *et al.* (2015).

Obviously, Heavy metals established naturally in the ecosystem with large variations in concentrations (Adeosun *et al.*, 2015). Most metals reach water streams originate from industrial, municipal and urban run-offs which can be deleterious to our life (Tolcin, 2011). Overwhelm civilization of industrialization could be the cause for an enhanced level of heavy metals in our waterways (Njar and Al-Doush, 2012). Heavy metals pervade in water as colloidal, particulate and dissolved phases (Adepoju-Bello *et al.*, 2009) with their existence in water bodies being either of natural origin (e.g. eroded minerals within sediments) or of anthropogenic origin (i.e. solid waste disposal, industrial or domestic effluents) (Marcovecchio *et al.*, 2007).

3. Correlation analysis:

Statistical analysis of variable (table, 3) between heavy metals pollution of milk and that of water indicated that there is a significant positive relationship ($P < 0.05$) between heavy metal pollution of milk and those in water samples. This mean that the main source of milk polluted with heavy metals is mainly originate from water contamination. Our result was coincided with (Rajaganapathy *et al.*, 2011; Mohod and Dhote, 2013).

CONCLUSION

From our obtained data, it can be concluded that lead and iron were more than the maximum permissible limit either in milk or groundwater. However, Cadmium less than the acceptable limits. Our data confirmed the high pollution of ground water sources and hence, they are not suitable for consumption without any prior treatment. Also, there is need for caution as they have the potential to bio-concentrate some of these heavy metals in food chain over time. So, we suggested that milk and ground water in Upper Egypt should be continuous precisely monitoring for other heavy metals pollution to obviate the risk of impact of heavy metals on human and animal health.

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تقييم التلوث بالمعادن الثقيلة في المياه الجوفية وحليب الابقار في صعيد مصر

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تناولت هذه الدراسة تقييم تلوث المعادن الثقيلة في المياه الجوفية وحليب البقر الخام في صعيد مصر .لذلك تم تجميع اجمالي عدد 102 عينه من المياه الجوفية وحليب الابقار من ثلاث محافظات في صعيد مصر، وهي أسيوط وسوهاج وقنا. وتم تقييم اربعه عناصر معدنيه في كل من المياه الجوفية وحليب الابقار باستخدام جهاز الامتصاص الذري شملت معدن الرصاص والكادميوم والحديد والمنجنيز. وأظهرت النتائج أن متوسط تركيز كل من الرصاص كان (٠,١٩٥ و ٠,٠٧٥ جزء في المليون) والحديد (٣,٩٤٩ و ٠,٨٦٩ جزء في المليون) والكادميوم (٠,٠٠٠٤٠ و ٠,٠٠٠٢٦ جزء في المليون) في عينات المياه الجوفية ولبن الابقار الخام على التوالي. وعلى النقيض من هذا لم نتمكن من الكشف عن وجود عنصر المنجنيز في أي من عينات المياه أو عينات الحليب. علاوة على ذلك أظهر التحليل الاحصائي لنتائج البحث أن هناك ارتباط معنوي ايجابي بين التلوث بالمعادن الثقيلة في إجمالي عينات المياه والحليب المختبره.

الكلمات الكاشفة: المعادن الثقيلة ، التلوث ، المياه الجوفية ، الألبان ، الأبقار.