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Lead, Cadmium and Copper Levels in Table Eggs

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ABSTRACT

A total of 100 fresh hen's egg samples (pooled samples) including farmers' houses and poultry farms' eggs were collected for estimation of lead, cadmium and copper levels (50 pooled samples for each type, each pooled was represented by 5 eggs). Poultry farms' egg samples were purchased from different shops and supermarkets, while farmers' houses eggs were purchased from farmers' houses in Assiut Governorate, Egypt. The samples were digested then, analyzed for detection and measurement of lead, cadmium and copper by using ZEE nit 700P Atomic Absorption Spectrophotometer with Graphite Furnace Unit (AASG) (Perkin –Elmer Atomic Absorption Spectrophotometry model 2380, USA). Results indicated that incidence of lead, cadmium and copper in farmers' houses egg samples were 96, 12 and 98% while in poultry farms' egg samples were 98, 32 and 100%, respectively. Moreover, the samples above MPLs for lead in farmers' houses eggs and poultry farms' eggs were 47 (94%) out of 50 examined samples and fortunately all of examined samples contained cadmium and copper below the MPLs (0%) as stated by EOSQC (1993).

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Introduction

Eggs are one of nature's most nutritious and economical foods in human daily diet and included in several food products for various functions. Hen eggs are important source of nutrients, containing all of proteins, lipids, vitamins and minerals. However, they are used as evidence for environmental pollution since they can accumulate the heavy metals from diet and the surrounding environment.

Poultry could take up heavy metal compounds from different sources; metal residues may concentrate in their meat, and eggs (Nisianakis *et al.*, 2009).

Heavy metals are the elements having density more than 5g/cm3, atomic weight 63.546 to 200.590 AMU (atomic mass unit) and a specific gravity greater than 4. Although heavy metals remain in ground water and soil but in certain areas their level increases and tends to accumulate to toxic levels in human and animal tissues deriving food from water and soils. Moreover, the industrial and agricultural processes have resulted in increased concentration of metals in air, water and soil and these metals are taken by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted water also accumulate such metals in their tissues (Yahaya *et al.*, 2010).

A large amount of these metals are taken by plants and animals and subsequently find their way into the food chain. This ever increasing pollution has given rise to concern on the intake of harmful metals that enter human body through inhalation, ingestion or absorption through the skin (Ogabiela *et al.*, 2010). Heavy metals can be classified as potentially toxic (cadmium, lead, etc.), probably essential (nickel, vanadium and cobalt) and essential (iron, manganese, copper, zinc and selenium) (Jalbani *et al.*, 2007). Toxic elements can be very harmful even at low concentration when ingested over a long period, especially lead (Pb) and cadmium (Cd) (Dundar and Deryaoglu, 2005). Lead is an environmental contaminant that occurs naturally and to a greater extent, from anthropogenic activities such as mining and smelting and battery manufacturing. It occurs in organic and inorganic forms; the latter predominates in the environment. Also, lead is found in tissues of animals and birds fed on contaminated plants and grains. Lead toxicities occurs when a bird inhales or ingests a concen-

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trated source of lead e.g. lead based paints, lead arsenate crop sprays, lead plates in automotive batteries, fishing sinkers, lead shotgun pellets, drapery weights, sewage sludge and lead mine tailing (Osweiler, 1996). Lead ingested by chicken is deposited in bones, soft tissues and eggs, so contaminated egg yolk represents a potential public health hazard especially to children repeatedly consuming eggs. Moreover, children have high gastrointestinal uptake and permeable blood-brain barrier. As lead can pass from hen to the egg, repeated consumption of contaminated eggs from a family owned flocks provide continuing dietary source of lead (Trampel *et al*., 2003). Cadmium is a toxic metal that has number of industrial applications as metal plating, pigments, rechargeable batteries and plastics. However, food is the primary source of cadmium exposure (WHO, 1992), and its adverse health effects occur in the form of kidney damage but possibly also bone effects and fractures (Järup, 2003). It also affects the cardiovascular system, hematopoietic cells, the immune and nervous system due to the occupational intoxication (Pan *et al*., 2010).

As copper (Cu) is an essential trace element, which is required by humans nevertheless, it is a normal constituent of animal tissues and fluids, crucial in hemoglobin synthesis and other enzymes functions. Both deficiency and excess of copper in the mammalian system are inconvenient effects (Hostynek *et al*., 1993). Toxic level of copper may lead to Wilson's disease (excessive accumulation of copper in liver, brain, kidney and cornea) and Menke's disease (peculiar hair, severe mental retardation, neurological impairment and death before 3 years of age) (Goyer, 1996). Consequently, the demand for organic food product has increased during the last decades due to their probable health effects (Soltoft *et al*., 2011).

The primary techniques for analyzing heavy metal in food samples are based on atomic absorption spectrometry, atomic emission spectrometry and mass spectrometry after digestion of organic material with concentrated acids. Atomic Absorption Spectrometry (GAAS) is a powerful detection technique for determining the trace elements (Leggli *et al*., 2010). Therefore this work was planned to estimate the level and risk of lead, cadmium and copper in hens' eggs collected from Assiut Governorate.

Materials and methods

Collection of samples

A total of 100 fresh hen's egg samples (pooled samples) including farmers' houses and poultry farms' eggs were collected for analysis (50 pooled samples for each type, each pooled was represented by 5 eggs). Poultry farms' egg samples were purchased from different shops and supermarkets, while farmers' houses eggs were purchased from farmers' houses. These samples were transferred in plastic bags to the laboratory and kept refrigerated at 4 ºC until analysis.

Preparation of samples

Sampled eggs were thoroughly rinsed in distilled water and diluted nitric acid to avoid external contamination, then sampled eggs were allowed to air dry. Each egg was punctured at broad end using clean and dried pointed forceps and dissecting scissors. The yolk and egg white of sampled eggs (5 eggs) were thoroughly blended in clean and dried glass beaker before weighting for analysis (Sobeih and Hegazy, 2011).

Microwave digestion

Microwave digestion procedure was applied for egg samples. Two grams of each sample were digested with 7 ml of HNO3 70% in microwave digestion system and diluted to 25 ml with deionized water. A blank digest was prepared in the same way but without adding egg samples and all sample solutions were clear and transparent (Soylak *et al*., 2005). Microwave condition system (Analytik Jena AG, 2008) was applied as one step at 190 ºC for 20 minutes.

Metal analysis of the samples was carried out in the Central Laboratory of the Faculty of Veterinary Medicine, Assuit University, Egypt. All samples, in addition to the blank were analyzed for detection and measurement of lead, cadmium and copper by using of ZEE nit 700P Atomic Absorption Spectrophotometer with Graphite Furnace Unit (GAAS) (Perkin-Elmer Atomic Absorption Spectrophotometer model 2380, USA). The analytical quality was maintained with repeated analysis of reference samples.

Quantitative determination of the studied metals

Concentrations of lead, cadmium and copper in the examined egg samples were calculated according to the following equation which described by Horwitz (2000).

$C = R \times D / W$

C: is the concentration of heavy metal (mg/kg) wet weight (ppm).

R: is the reading of element concentration on digital scale of Atomic Absorption Spectrophotometer.

D: is the final volume of prepared sample in ml.

W: is the weight of the wet sample.

Concentrations of metals were expressed as mg/kg of the sample based on wet weight. The concentration of absorbance values of metals in the blank samples were also calculated and subtracted from each analyzed sample to exclude any traces of metal that might be present in the used acids for digestion.

Results

Results achieved in Table 1, deonstrated that 48 (96%) out of 50 examined egg samples obtained from farmer's houses eggs were positive for lead and it's level ranged from 0.045 to

Table 1. Occurrence and levels of lead, cadmium and copper in examined farmers' houses eggs samples (mg/ kg)

Element	No. of examined samples	Detectable samples		Concentration (mg/kg)		
		No.	%	Min.	Max.	Mean ± SE
Lead	50	48	96	0.045	1.330	0.64±0.05
Cadmium	50	6	12	0.002	0.006	0.004±0.001
Copper	50	49	98	0.060	1.120	0.49±0.004

Data are presented as mean ± SE

No.: Number, Min.: Minimum, Max.: Maximum

1.330 mg/kg with a mean value of 0.64±0.05mg/kg.

In case of poultry farms egg samples, 49 (98%) were positive for lead. Lead levels of examined samples ranged from 0.0048 to 1.15 mg/kg with a mean value of 0.59 ±0.03 mg/kg (Table 2).

The obtained results in Table 3 revealed that 47 (94%) and 48 (96%) out of 50 examined samples were above the MPLs for lead while all examined samples contained cadmium and copper below the MPLs as stated by EOSQC (1993).

Table 4 revealed that the average concentration of lead, cadmium and copper in the examined table egg sample were 0.615, 0.012 and 0.530 mg/kg,

Table 5 showed the comparison of standard lead, cadmium and copper intakes with the estimated intakes in farmers' houses egg samples (mg/kg/b.w.) according to CAC (2013.

Table 6 summarized the comparison of standard lead, cadmium and copper intakes with the estimated intakes in poultry farms' egg samples (mg/ kg/ b.w.): according to CAC (2013).

Table 2. Occurrence and levels of lead, cadmium and copper in examined poultry farms' egg samples (mg/ kg)

Element	No. of examined samples	Detectable samples		Concentration (mg/ kg)		
		No.	%	Min.	Max.	Mean ± SE
Lead	50	49	98	0.0048	1.15	0.59±0.03
Cadmium	50	16	32	0.0015	0.07	0.02±0.004
Copper	50	50	100	0.0400	1.90	0.57±0.04

Data are presented as mean ± SE

No.: Number, Min.: Minimum, Max.: Maximum

Table 3. Percentage of samples above the maximum permissible limits in farmers' houses and poultry farms' eggs according to EOSQC (1993)

Element	Examined samples	Standard of MPL (mg/ kg)	Samples above MPL			
			Farmers' houses samples		Poultry farms samples	
			No.	%	No.	%
Lead	50	0.1 mg / kg	47	94	48	96
Cadmium	50	0.1 mg / kg	0	0	0	0
Copper	50	2 mg / kg	0	0	0	0

MPL= maximum permissible limit

Table 4. Comparison of Acceptable Daily Intake (ADI) value of heavy metals with calculated daily intake of metals from all examined egg

Element	ADI mg/ 70 kg person (a)	Average of metal in total examined egg samples	Calculated average daily intake of metals from consuming100g/egg/day (b)	
			mg/day/person	%
Lead	0.0036	0.615	0.0615	17.8
Cadmium	0.0010	0.012	0.0012	120
Copper	35.0000	0.530	0.0530	0.15

a: According to Codex Alimentarius Commission (2013).

b: According to Nutritional Institute, Egypt (2006).

Table 5. Comparison of standard lead, cadmium and copper intakes with the estimated intakes in farmers' houses egg samples (mg/kg/b.w.) according to CAC (2013)

Element	DI				WI			
	ADI	EDI	Samples above ADI		PTWI	EWI	Samples above PTWI	
			No.	%			No.	%
Lead	0.0036	0.9100	48	96	0.025	6.3700	48	96
Cadmium	0.001	0.0057	6	12	0.007	0.0399	6	12
Copper	35.0	0.7000	0	0	245	4.9000	0	0

DI = Daily intake
ADI = Acceptable daily intake
EDI = Estimated daily intake
WI = Weekly intake
PTWI = Provisional tolerable weekly intake
EWI = Estimated weekly intake

Table 6. Comparison of standard lead, cadmium and copper intakes with the estimated intakes in poultry farms' egg samples (mg/ kg/ b.w.): according to CAC (2013)

Element	DI				WI			
	ADI	EDI	Samples above ADI		PTWI	EWI	Samples above PTWI	
			No.	%			No.	%
Lead	0.0036	0.840	49	98	0.025	5.880	49	98
Cadmium	0.0010	0.028	16	32	0.007	0.196	16	32
Copper	35.0000	0.800	0	0	245	5.600	0	0

DI = Daily intake
ADI = Acceptable daily intake
EDI = Estimated daily intake
WI = Weekly intake
PTWI = Provisional tolerable weekly intake
EWI = Estimated weekly intake

Discussion

From the results achieved in Table 1, it is evident that 48 (96%) out of 50 examined egg samples obtained from farmer's houses eggs were positive for lead and it's level ranged from 0.045 to 1.330 mg/kg with a mean value of 0.64±0.05mg/kg. Higher incidence was demonstrated by Chowdhury *et al.* (2011), however, Sobeih and Hegazy (2011); Al-Ashmawy (2013) estimated lower levels. In contrast, lead could not be detected in the examined samples of local breed eggs as noticed by Surtipanti *et al.* (1995). High level of lead in farmers' houses eggs may be attributed to the feeding behavior of these hens as they forgoing in soil of house which may contain high level of lead. In case of poultry farms egg samples, 49 (98%) were positive for lead. Lead levels of examined samples ranged from 0.0048 to 1.15 mg/kg with a mean value of 0.59 ±0.03 mg/kg (Table 2). Similar lead levels were detected by Fakayode and Olu-Owolabi (2003); Khan and Naeem (2006) however, lower levels were estimated by Sobeih and Hegazy (2011); Hashish *et al.* (2012); Al-Ashmawy (2013); Basha *et al.* (2013); Shahid ul Islam *et al.* (2014). On the other hand, Chowdhury *et al.* (2011) detected higher level than the obtained results.

Regarding to data presented in Table 1, it is evident that 6 (12%) out of 50 examined farmer's houses eggs samples were positive for cadmium. In addition, cadmium levels ranged from 0.002 to 0.006 mg/kg with a mean value of 0.004 ± 0.001mg/kg. Cadmium was not detected by Sobeih and Hegazy (2011); Al-Ashmawy (2013), but the results of this study were lower than those levels detected by Chowdhury *et al.* (2011). While, 16 (32%) poultry farms egg samples were positive for the presence of cadmium. Moreover, cadmium level of the examined samples ranged from 0.0015 to 0.07mg/kg with a mean value of 0.02 ±0.004 mg/kg (Table 2). Lower levels were estimated by AbdulKhaliq *et al.* (2012) ; Basha *et al.* (2013), while, Chowdhury *et al.* (2011) indicated higher findings. In contrast, cadmium was not detected in the samples evaluated by Sobeih and Hegazy (2011); Al-Ashmawy (2013); Shahid ul Islam *et al.* (2014).

In the present study, the low level of cadmium may be due to very low transfer dietary of cadmium to the eggs as indicated by Leach *et al.* (1979). Fortunately, the obtained cadmium concentration was below the limit of quantification, which in harmony with that detected by Waegeneers *et al.* (2009); Hashish *et al.* (2012).

Concerning copper, 49 (98%) out of 50 examined farmers' houses eggs samples were positive in a level ranged from 0.060 to 1.120 mg/kg with a mean value of 0.49 ± 0.004 mg/kg (Table 1). These results were lower than that postulated by Chowdhury *et al.* (2011); Sobeih and Hegazy (2011); Al-Ashmawy (2013). As low level of copper could be attributed to its deficiency in the hen's diet or due to other adverse environmental condition.

Regarding poultry farm egg samples all of them were positive in a mean value of 0.57 ±0.04 mg/kg as shown in Table 2 which nearly similar to that obtained by Hashish *et al.* (2012) ; Al-Ashmawy (2013); Basha *et al.* (2013) ; Shahid ul Islam *et al.* (2014). However lower levels were estimated by Chowdhury *et al.* (2011); AbdulKhaliq *et al.* (2012).

The obtained low levels of copper may be attributed to that the eggs considered poor sources of copper as demonstrated by Jeng and Yang (1995) and Amer *et al.* (2006). Copper is stored in liver, bone and bone marrow where it bound to metallothionine as reported by Sarkar *et al.* (1983) which explain its low levels in table eggs.

The maximum permissible limit (MPL) for lead and cadmium in egg samples should not exceed 0.1mg/kg and 2 mg/kg for copper according to EOSQC (1993). Researches didn't find special MPL in eggs, so this research takes MPL of food grade. The obtained results in Table 3 revealed that 47 (94%) and 48 (96%) out of 50 examined samples were above the MPLs for lead while all examined samples contained cadmium and copper below the MPLs as stated by EOSQC (1993). The high percentage of samples which contained lead above MPL may be attributed to the continual increase in the number of industrial and agricultural processes producing these pollutants.

Table 4 showed that the average concentration of lead, cadmium and copper in the examined table egg sample were 0.615, 0.012 and 0.530 mg/kg, daily intake 0.0615, 0.0012 and 0.0530 mg/day/person representing about 17.8, 120 and 0.15 % of ADI (Codex Alimentarius Commission, 2013).

Average daily intake of lead, cadmium and copper as revealed in this study were higher than that demonstrated by Sobeih and Hegazy (2011) and Al-Ashmawy (2013). These higher findings may occur as result of the environmental pollution with these metals in air, water, soils, feeds and finally to birds which pass to eggs.

As recorded in Table 5, the EDI and EWI of lead were 0.91 and 6.37 mg/kg b.w., respectively, which were above ADI and PTWI as stated by CAC (2013). 48 (96%) each of egg samples above ADI and PTWI were above ADI and PTWI as recorded by CAC (2013).

Likewise, the EDI of cadmium was 0.0057 mg/kg b.w. and 6 (12%) of farmer's houses egg samples were above ADI and the EWI was 0.0399 mg/kg b.w. and 6 (12%) of egg samples were above PTWI that recorded by CAC (2013). While, the EDI and EWI of copper were 0.70 and 4.9 mg/kg b.w., respectively and none of egg samples were above ADI. Moreover, the average of EDI and EWI of copper were lower than ADI and PTWI as stated by CAC (2013).

Data summarized in Table 6 revealed that, the EDI and EWI of lead were 0.84 and 5.88 mg/kg b.w., respectively, which were above ADI and PTWI. 49 (98%) each of egg samples above ADI and PTWI were above ADI and PTWI as recorded by CAC (2013).

The EDI of cadmium was 0.028 mg/kg b.w. and 16 (32%) of poultry farm's egg samples were above ADI and the EWI was 0.196 mg/kg b.w. and 16 (32%) of samples were above PTWI that recommended by CAC (2013). While, the EDI and EWI of copper were 0.8 and 5.6 mg/kg b.w., respectively and none of egg samples were above ADI. The average of EDI and EWI of copper were lower than ADI and PTWI (CAC, 2013).

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