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Assessment of levels and health risk of heavy metals (Pb, Cd, Cr, and Cu) in commercial hen's eggs from the city of Hamedan

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ABSTRACT: Increasing consumption of chicken products in Iran makes it very important to analyze their residual heavy metal contents; therefore, the present study has been conducted to determine Pb, Cd, Cr, and Cu in commercial hen eggs, marketed in the city of Hamedan in 2016. In this descriptive study, a sum of 27 samples of hen eggs has been collected from the Hamedan City's market. After preparation and processing the samples in the laboratory, the concentration of metals has been determined in mixed albumen and yolk, using inductively coupled plasma-optical emission spectrometry. Also, all statistical analyses have been conducted, using the SPSS statistical package (version 20) with the results showing that the mean concentrations (mg/kg) of Pb, Cd, Cr, and Cu in the samples have been 0.29±0.16, 0.18±0.04, 0.31±0.03, and 2.81±1.56, respectively. Also, the mean contents of Cd and Cr have surpassed the maximum permissible levels (MPL), established by WHO/FAO. The computed health risk index values show that there is no potential risk for adults and children through egg consumption at the current rate in the study area. According to the results, considering the mean contents of Cd and Cr observed in egg samples have been higher than the MPL; therefore, it is recommended to pay serious attention to pollutants discharge in the environment and monitor chemical residue, especially heavy metals, in the foodstuff.

Keywords: adverse health effect, chicken product, food safety, heavy metals, ICP-PES.

INTRODUCTION

Human activities such as rapid industrialization and urbanization, mining, fossil fuel combustion, smelting, and refining nonferrous metals, as well as spread of sewage sludge over farmlands can release pollutants, especially toxic heavy metals, into the environment (Jarup & Akesson, 2009).

Since some major characteristics of heavy metals such as their long biological half-life, endurance, and non-biodegradability, can cause them get accumulated within soil-plant-food chains. As such, presence of these metals in considerable amounts in the environment indicates a potential health risk for human along with the environment (Rezaei Raja et al., 2016; Sobhanardakani et al., 2017).

It has been proven that exposure to heavy metals and their accumulation in human body starts at an early age via food consumption as the main source (Schoeters et al., 2006; Bernard, 2008). Also, other culprits of human exposure to heavy metals include smoking, air, water, and the occupational environment (Zhang et al., 2017).

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Lead is known for its toxic and adverse health effects on public health, with both air and food being the commonest ways of population's exposure to Pb. Ingested by chicken via contaminated feed, this metal is deposited in soft tissues, bones, and eggs. As lead can be sequestered from hen to egg, repeated consumption of contaminated eggs provide a continuing dietary source of lead. Development of abnormalities, deficits in intelligence quotient and neurotoxicity effects in infants, incidence constipation, colic, and anemia are the main consequences of chronic exposure to Pb (Duran et al., 2009; Iwegbue, 2011; Hariri et al., 2015).

Cadmium has an exceptionally long biological half-life between 10 and 30 years; consequently, it can be harmful to human, particularly through its accumulation in the kidneys (Bernard, 2008; Jarup & Akesson, 2009; Nookabkaew et al., 2013; Kim et al., 2014; Sobhanardakani et al., 2015). It is also listed as a human carcinogen in Group 1 by the IARC. Decreased rate of glomerular filtration, significant proteinuria, and increased frequency of kidney stone formation are the chronic effects of oral exposure to this metal (Zhang et al., 2017). Low-level postnatal Cd exposure may cause neurotoxic effects in children (Ciesielski et al., 2012; Llop et al., 2013; Rodriguez-Barranco et al., 2013; 2014; Silver et al. 2013).

Chromium is an essential element for human beings, especially since it acts on the organism, maintaining normal glucose tolerance. Chromium (III), found in most food and nutrient supplements, is an essential nutrient with very low toxicity, whereas Cr (VI) compound have been shown to be potent occupational carcinogens. Stainless steel vessels seem to be the main source of this element's contamination (Upreti et al., 2004).

Copper, as an essential trace element, also plays a variety of roles: It is crucial in hemoglobin synthesis and enzyme functions, while being the normal constituent of animal tissues and fluids. Although both deficiency and excess of this element in the human body result in adverse effects, the toxic level of Cu may lead to Menkes's and Wilson's diseases (Tapiero & Tew, 2003; Sobhanardakani et al., 2014).

Poultry could take up heavy metal from different sources, especially via nutrition. Therefore, metal residues may concentrate in their meat, and eggs (Sparks, 2006; Nisianakis et al., 2009; Chowdhury et al., 2011; Abdulkhaliq et al., 2012). Since hen eggs are considered as one of nature's highly-nutritious and economical food items in human daily diet, especially that of the children, it is of high account for human health (Surai & Sparks, 2001; AL-Ashmawy, 2013).

The human health risk assessment requires identification, collection, and integration of information on hazardous chemicals, their exposure to humans, and also the relationship between exposure, dose, and adverse health effects (Sobhanardakani, 2017).

Eggs are generally consumed by members of different income classes; thus this product's contamination can cause problems to consumers. Since knowledge of eggs' metal levels is becoming increasingly important and egg consumption is a bioindicator to monitor environmental pollution (Surai & Sparks, 2001; Sparks, 2006; Pappas et al., 2006), the current study has attempted to analyze the contents and risks, associated with Pb, Cd, Cr, and Cu, through regular consumption of hen eggs in the city of Hamedan in 2016.

MATERIAL AND METHODS

In this study, 27 samples of hen's eggs were purchased from different markets of Hamedan and used to analyze the concentration of Pb, Cd, Cr, and Cu.

Standard stock solutions of different metal ions at the concentration of 1000 μg/mL were used to prepare working solutions after appropriate dilution. Standard solutions were of analytical grade (Sigma-Aldrich, Spain). Distilled deionized water was used in all dilution procedures.

For sample analyses, each egg was washed with de-ionized water and then its white and yolk were mixed in a 200 ml beaker (Uluozlu et al., 2009). Five grams of the mixed sample were digested with 6 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) (Merck, Germany) into 100 ml beaker and covered with a watch glass. After 30 min the beaker was placed on a hot plate up to 140 °C until the complete decomposition of the sample was achieved and the total volume was reduced to nearly 3 ml. the digested sample was cooled and filtered into a 50 ml calibrated flask with double-distilled water using Whatman 42 filter paper (Waegeneers et al., 2009; Leggli et al., 2010). Finally, for Pb, Cd, Cr, and Cu analyses with three replications, the present study employed a Varian710-ES, inductively coupled with plasma-optical emission spectrometry (wave length for Pb, Cd, Cr, and Cu were 220.35 nm, 226.50 nm, 267.72 nm, and 324.75 nm, respectively).

The statistical analysis of the obtained results initially consisted of Shapiro-Wilk test for normality. The mean levels of heavy metals were compared with maximum permissible limits, using a one-sample test. Finally, to study the correlation between the metals in different egg samples, the 2-tailed test of Pearson correlation was taken.

For potential health risk assessment, in the first step human health risks, posed by chronic exposure to heavy metals, were evaluated in accordance with Equation (1) (Guo et al., 2016).

For this purpose the () was calculated using

$$DIM = \frac{Cmetal \times Cfactor \times Dfood\ intake}{Baverage\ weight} \quad (1)$$

where DIM represents the average daily intake of metal (mg), C_{metal}, C_{factor}, and $D_{food\ intake}$, stand for heavy metal levels in the analyzed eggs (mg/kg), conversion factor (0.085), and egg daily intake (3.0e0-2 kg per person per day), respectively. Also, $B_{average\ weight}$ is the average body weight, which is 70.0 kg for adults and 15.0 kg for children (Falco et al., 2006; Omar et al., 2013; Tang et al., 2015).

In the second step, the Health Risk Index (HRI) for local population through egg consumption was assessed, using the Equation (2) (Guo et al., 2016).

$$HRI = \frac{\text{DIM}}{\text{RfD}} \quad (2)$$

where DIM and RfD indicate the daily metal intake (mg) and reference dose of the metal (mg/kg/day), respectively. The oral reference doses were 0.0035, 0.001, 1.50, and 0.04 for Pb, Cd, Cr, and Cu, respectively. When below 1, HRI means that the exposed population is assumed to be safe (Sobhanardakani, 2017).

Total HRI (THRI) of heavy metals for the eggs was calculated, according to Equation (3) (Guo et al., 2016).

$$\text{THRI} = \text{HRI}(\text{toxicant1}) + \text{HRI}(\text{toxicant2}) + \ldots + \text{HRI}(\text{toxicant n}) \quad (3)$$

RESULTS AND DISCUSSION

Table 1 present the contents of Pb, Cd, Cr, and Cu in the analyzed samples, showing that among the analyzed samples, Pb ranged from 0.10 to 0.60 mg/kg; Cd between 0.10 and 0.30 mg/kg; Cr from 0.10 to 0.50 mg/kg; and Cu between 1.0 and 6.0 mg/kg.

Based on the comparison of independent one-sample t-test, the heavy metal contents in egg samples with the maximum permissible limits (mg/kg) (i.e. 0.50, 0.05, 0.002, and 10.0 for Pb, Cd, Cr, and Cu, respectively), established by FAO/WHO (Roychowdhury et al., 2003), shows that the mean contents of Cd and Cr, determined in all samples, were higher than the MPL.

The Pearson's correlation analyses were conducted among metal concentrations in egg samples to understand the relations among them. According to the results, there was not any significant correlation between Pb and Cd (r= 0.271, *P*= 0.171), Pb and Cr (r = 0.297, *P*= 0.132), Cd and Cr (r = 0.035, *P*= 0.863), Cd and Cu (r = 0.290, *P*= 0.142), and Cr and Cu (r = 0.366, *P*= 0.060). However, results showed that there was a significant positive correlation between the concentrations of Pb and Cu (r = 0.858, *P*< 0.010).

In addition, assuming consuming the weekly rate of three eggs per person, all calculated HRI values of heavy metals stayed within the safe limits (HRI < 1) (Table 2). Furthermore, THRI values, in egg samples, varying between 5.59e-03 and 2.26e-02 for adults and between 2.61e-02 and 1.06e-01 for children, were also within the safe limit (THRI < 1). Therefore, it can be concluded that people might face no potential significant health risk through mere consumption of the analyzed eggs, under the current consumption rate.

Table 1. Residual levels of examined heavy metals in egg samples (mg/kg, wet weight)

Metal	Positive samples (>LOD)		Min.	Max.	Mean	S.D
	No.	%				
Pb	26	96.30	0.10	0.60	0.29	0.16
Cd	27	100.0	0.10	0.30	0.18	0.04
Cr	27	100.0	0.10	0.50	0.31	0.03
Cu	26	96.30	1.00	6.00	2.81	1.56

Table 2. Daily intakes of metals (DIM, mg) and health risk index (HRI) for individual heavy metal, caused by the analyzed eggs

	Pb	Cd	Cr	Cu
Adults				
DIM	1.06e-05	6.56e-06	1.13e-05	1.02e-04
STD	5.83e-06	1.46e-06	1.09e-06	5.68e-05
Min	3.64e-06	3.64e-06	3.64e-06	3.64e-05
Max	2.18e-05	1.09e-05	1.82e-05	2.18e-04
HRI	3.02e-03	6.56e-03	7.53e-06	2.56e-03
STD	1.66e-03	1.46e-03	7.28e-07	1.42e-03
Min	1.04e-03	3.64e-03	2.43e-06	9.11e-04
Max	6.24e-03	1.09e-02	1.21e-05	5.46e-03
Children				
DIM	4.93e-05	3.06e-05	5.27e-05	4.78e-04
STD	2.72e-05	6.80e-06	5.10e-06	2.65e-04
Min	1.70e-05	1.70e-05	1.70e-05	1.70e-04
Max	1.02e-04	5.10e-05	8.50e-05	1.02e-03
HRI	1.41e-02	3.06e-02	3.51e-05	1.19e-02
STD	7.77e-03	6.80e-03	3.40e-06	6.63e-03
Min	4.86e-03	1.70e-02	1.13e-05	4.25e-03
Max	2.91e-02	5.10e-02	5.67e-05	2.55e-02

Heavy metal toxicity has proven to be a major threat and with several health risks associated with it (Jaishankar et al., 2014).

Lead as a toxic element can damage intellectual performance, resulting in reduced cognitive development in children. It also causes cardiovascular disease and increased blood pressure in adults (Uluozlu et al., 2009). In the current study, the highest and lowest Pb levels (mg/kg) in egg samples were found 0.10 and 0.60, respectively. In this regard, the mean levels of Pb (mg/kg) in egg albumen from Markazi Province (Iran) was reported within the range of 0.225-5.363 (Farahani et al., 2015). Khan and Naeem (2006) reported that Pb levels of eggs from Pakistan ranged between 0.52-0.63 mg/g. Also, Fakayode and Olu-Owolabi (2003) studied the content of Pb (mg/g) in eggs from Nigeria and reported that the overall average concentration for Pb was 0.59. Table 3 compares the results of this study with other studies.

Table 3. Comparison of metal contents (mg/kg) in egg samples with the values, reported in other studies

Location	Pb	Cd	Cr	Cu	Source
Egypt	-	-	-	6.32	AL-Ashmawy (2013)
Palestine	0.27	0.036	-	2.70	Abdulkhaliq et al. (2012)
Nigeria	0.80	0.18	0.10	1.03	Iwegbue et al. (2012)
Bangladesh	1.06	0.08	-	0.26	Chowdhury et al. (2011)
United Kingdom	0.24	-	0.08	-	Siddiqui et al. (2011)
Greece	-	0.001	0.06	0.78	Giannenas et al. (2009)
Turkey	0.06	2.34	0.04	0.45	Uluozlu et al. (2009)
Belgium	0.095	0.0005	-	0.48	Waegeneers et al. (2009)
Belgium	0.009	0.0003	-	0.51	van Overmeire et al. (2006)
France	0.01	0.0004	-	0.59	Leblanc et al. (2005)
Denmark	< 0.005	< 0.0006			Larsen et al. (2002)
United Kingdom	0.003	0.0004	-	0.62	Ysart et al. (2000)
This study	0.29	0.18	0.31	2.81	

Cadmium may accumulate in human body, likely to cause reproductive deficiencies, skeletal damage, and kidney dysfunction (Uluozlu et al., 2009). The minimum and maximum cadmium contents (mg/kg) of the egg samples were found 0.10 and 0.30. Also, according to the results the mean contents of Cd in egg samples were higher than MPL. The main source of Cd contamination can be attributed to the use of chemical pesticides to cultivate poultry food as well as poultry medicines, containing cadmium (Farahani et al., 2015). Cadmium levels in egg samples have been reported within the range of 0.025-6.388 mg/kg in eggs albumen in Iran (Farahani et al., 2015), while it ranged between 0.07 and 0.08 mg/g in Pakistan (Khan & Naeem, 2006), and around 0.07 mg/g in Nigeria (Fakayode & Olu-Owolabi, 2003). Table 3 compares Cd levels (mg/kg) in eggs with those of other studies.

Chromium, particularly Cr (III), only in trace amount plays an important role in metabolic functions, and cofactor of insulin, but can be toxic when it exceeds the maximum permissible limit (Chowdhury et al., 2011). It should be noted that oxidation state and solubility are the major factors, governing the toxicity of chromium compounds (De Flora et al., 1990). According to the results of this study, the mean contents (mg/kg) of Cr in the egg samples were 0.31±0.03, higher than MPL. This can be attributed to the ingestion of food and water that contain Cr through processing and preparation by poultry (Langard & Vigander, 1983). In other studies, Dobrzanski et al. (2007) reported that Cr contents (mg/g) in the various egg components were in the range of 0.021-1.173. Table 3 compares the results of this study with other studies.

It has been proven that Cu has both toxicity and vital effects for many biological systems and may enter food materials from soil in several ways such as mineralization by crops, food processing, or environmental contamination, as in the application of agricultural inputs, including chemical fertilizers and also copper-based pesticides (Onianwa et al., 2001; Koc et al., 2008; Uluozlu et al., 2009). In the current study, the mean concentrations of Cu (mg/kg) in egg samples were 2.81±1.56, lower than MPL. The mean contents of Cu in eggs in the literature is reportedly 0.07 mg/100g in Brazil (Ferreira et al., 2005), 1.13 mg/kg in Nigeria (Onianwa et al., 2001), 0.64 mg/kg in USA (Pennington et al., 1995), and 0.80 mg/kg in USA (Lurie et al., 1990). Table 3 compares Cu levels (mg/kg) in eggs with those of other studies. Results show that Cu values in egg samples surpassed most literature values.

According to the analysis of health risk index values (Table 2), HRI values of Pb, Cd, Cr, and Cu for children and adults were below 1. Here, the average HRI value in egg samples was 3.04e-03 for adults and 1.42e-02 for children; therefore, it can be concluded that a target population might face no significant potential health risk only by consuming the analyzed eggs at the current consumption rate within the study area. However, non-carcinogenic risks were greater for children than adults.

CONCLUSION

Toxic heavy metals can have serious adverse impacts on human health. For this reason, the present investigation is mainly focused on the evaluation of Pb, Cd, Cr, and Cu in egg samples, collected from the city of Hamedan. According to the results, even though THRI values for individual metals in all samples were below 1 (within safe level), since the mean contents of Cd and Cr, observed in all samples exceeded the maximum allowed values, long-term consumption of egg especially by children can lead to chronic health effects. Therefore, it is recommended to pay serious attention to pollutant discharge into the environment; monitor chemicals residue, especially toxic heavy metals in the foodstuffs; control heavy metals content during the whole production process of poultry foods; and research on the effects of hen diet supplementation with various metal contents.

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