ESSENTIAL AND TOXIC METALS CONTENT IN HEN EGGS AT DIFFERENT GEOGRAPHIC AREA IN BOSNIA AND HERZEGOVINA

Vehab, Aida[1]**, Huremović,** Jasna[1]**, Žero,** Sabina[1] and **Šabanović,** Elma[1]

[1]*Department of Chemistry, Faculty of Science, University of Sarajevo, ZmajaodBosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina*
*Corresponding Author: jasnahuremovic@yahoo.com

Abstract
Hen eggs are an important part of diet for its nutrient value as well as the different possibilities of preparation and often represent the main source of animal protein. The aim of this study was to determine the content of essential and toxic metals in egg albumen and egg yolk produced from home-grown hen and farms in Bosnia and Herzegovina. The content of calcium, magnesium, zinc, iron, manganese, chromium, lead and cadmium in hen eggs was determined by using flame atomic absorption spectrometry (FAAS). Results showed that among the examined essential metals, calcium occurred at the highest concentrations, especially in the hen egg yolks.The most significant difference in concentrations was obtained for iron in hen egg albumens at different sampling sites.Minimum difference in concentrations of examined metals among the farm and home-grown samples was obtained for manganese. Obtained results have been compared with the content of heavy metals in hen eggs from different countries. In addition, the acceptable daily intakes of Cd, Cr, Fe, Mn, Pb and Zn were calculated. The highest (Zn) and the lowest (Cd) average daily intake from consuming of 100 g egg/day were obtained for home-grown hen eggs. Average daily intake of Fe and Cd from consuming of 100 g egg/day from farm hen eggs had the highest and the lowest value, respectively. Eggs are a notable essential elements source and the consumption of eggs does not necessarily mean a considerable contribution of toxic metals.

Keywords: metals, hen eggs, acceptable daily intake, FAAS

Received: 08.01.2019 Reviewed: 08.03.2019 Accepted: 13.03.2019

INTRODUCTION

Large quantities of pollutants have continuously been introduced into ecosystems as a consequence of urbanization and industrial processes. Heavy metals contamination is a major problem of our environment (Bukvić *et al.*, 2013). Global environmental pollution through heavy metal leads to an increased interest in metals contamination of food. Eggs symbolize an important part of human´s diet especially children due to their important role as source of nutrients, containing all of proteins, lipids, vitamins and minerals (Mora, 2003; Abduljaleel and Shuhaimi-Othman, 2011). Fresh eggs are among the most important and nutritious food in daily diet. Moreover, eggs are included in many food products for different functions (AL-Ashmawy, 2013; Ul Islam *et al.*, 2014). The contamination of food products by environmental contaminants becomes more and more documented. In general, data about contaminants in food are gathered to obtain information for background concentrations in terms of intake and associated risk assessments (Hashish *et al.*, 2012). However, eggs might contain elevated levels of heavy metals that originate mainly from food and water (Abdulkhaliq *et al.*, 2012) Metals are found in all living organisms having different roles. Metals such as Fe, Cu, Mg, Co, Zn are essential for human body but chronic metabolic disturbances may occur due to the lack or excess of these metals. It is important to maintain proper range of content of these metals for establishment of normal metabolic functions in human body (Chowdhury *et al.*, 2011). Poultry farming is being concentrated around the large urban centres all over the country. All mineral elements present in poultry feed at higher amounts can cause several adverse effects to poultry products (Ul Islam *et al.*, 2014). Hen egg might contain higher levels of

heavy metals than originate from food and water mostly because of the surroundings (Basha *et al.*, 2013). Hen egg naturally contains some metals and minerals. Mineral requirements of laying hens have to be met for optimum egg production (Demirulus, 2013).
In this study the content of Ca, Cd, Cr, Fe, Mg, Mn, Pb and Zn in hen egg yolk and albumen was determined by flame atomic absorption spectrometry (FAAS). A comparison of the metal content in hen eggs from home-grown hens and from the farm was reported. Also, obtained results have been compared with the content of heavy metals in hen eggs from different countries.The acceptable daily intakes of Cd, Cr, Fe, Mn, Pb and Zn were calculated.

MATERIALS AND METHODS

Standard solutions of Ca, Cd, Cr, Fe, Mg, Mn, Pb and Zn were prepared by diluting respective single element atomic absorption stock solution (1000 mg/L, Certipur grade, Merck, Germany). Dilutions were made by using Milli–Q water. All chemicals were of analytical reagent grade. Suprapur grade nitric (65%) and sulphuric (96%) acid(Merck, Germany) were used for the digestion of samples. Before the use, all the glass containers were cleaned by soaking in dilute nitric acid and rinsed by Milli–Q water.
Atomic absorption spectrometer, Varian model AA 240 FS, equipped with hallow cathode lamps for Ca, Cd, Cr, Fe, Mg, Mn, Pb and Zn was run under the conditions recommended by the manufacturer. Additional instruments used for the experimental part of this work included an analytical balance with precision ± 0.1 mg (Mettler – Toledo, USA) and a dryer (Bodalec and Havoić apparatus).

Sampling

Samples of hen eggs were taken from different locations in Bosnia and Herzegovina, including Sarajevo, Vogošća, Kakanj, Visoko and Tešanj. Specimens of home-grown hen eggs, where only natural food was used for feeding hens, without any supplements (5 locations) and egg samples (farm eggs) obtained from breeding hens on farms where mandatory food was supplemented by additions such as concentrate (5 farms). Ten eggs were collected from each location in February, 2015 (Table 1).

Table 1: Sample marks (HA- Home-grown egg albumen, HY- Home-grown egg yolk, FA- Farm egg albumen, FY- Farm egg yolk) and sampling locations

Sample	Home-grown hen eggs		Sampling location
	Albumen acronym	Yolk acronym	
1.	1HA	1HY	Obre-Kakanj
2.	2HA	2HY	Vehabi-Kakanj
3.	3HA	3HY	Hrasno-Kakanj
4.	4HA	4HY	Brežani-Kakanj
5.	5HA	5HY	Vogošća
Sample	Farm hen eggs		Sampling location
	Albumen acronym	Yolk acronym	
1.	1FA	1FY	Sarajevo
2.	2FA	2FY	Vehabi-Kakanj
3.	3FA	3FY	Doboj-Kakanj
4.	4FA	4FY	Tešanj
5.	5FA	5FY	Visoko

Determined content of the analytes in egg samples as well as the daily intake from eggs were compared with the World Health Organization (WHO) and Food Agriculture Organization (FAO) data of acceptable daily intakes (ADI) for humans (FAO/WHO, 1972, 1974c, 1982, 1987, 1989).
Sample preparation and chemical analysis
Hen egg albumen and yolk were separated. Ten albumen and yolk samples from the same sampling location were pooled and homogenized respectively by electrical plastic

mixer and then stored in clean polyethylene bottles at -20 °C until measurement.

One gram of sample (albumen and yolk) was weighed and dried at 75 °C for one hour. Dried samples were dissolved by the mixture of 20 mL HNO_3 and 10 mL H_2SO_4 (one sample per one Teflon container). Containers were allowed to stand one hour at the fume hood so that the fumes of nitrogen oxides evaporate. Further, containers were sealed and gently heated on a hot plate until the samples were completely dissolved. The samples were diluted by Milli-Q water, filtered using quantitative filter paper into a 100 mL volumetric flask and filled up by Milli-Q water to the mark and then analysed by FAAS.

Quality assurance

The quality assurance of the procedure for the estimation of essential and toxic metals in egg samples by FAAS has been assessed by calibrating the instrument using a mixture of respective single element atomic absorption standard solutions. Linear calibration graphs with correlation coefficients > 0.999 were obtained for all metals. It indicates that the method has a good precision for tested metals. The method validation was further ascertained by spike recovery and replicate analysis, calculating the standard deviation. The relative standard deviation between analyses was <10 %.

The limits of detection (LOD) and quantification (LOQ) for eight metals were calculated as three times and ten times the standard deviation of twelve replicate measurements of reagent and procedural blank sample, respectively, and the results are present in Table 2.

Table 2: Limit of detection (LOD), limit of quantification (LOQ) and spikes recovery (%) of the determined metals

Metal	Limit of detection (mg/L)	Limit of quantification (mg/L)	Spike recovery (%)
Ca	0.08	0.30	98-102
Mg	0.02	0.04	96-102
Zn	0.009	0.02	93-97
Pb	0.03	0.10	93-100
Cd	0.002	0.007	95-103
Cr	0.05	0.20	90-97
Fe	0.09	0.30	92-97
Mn	0.06	0.18	99-103

Acceptable daily intake (ADI)

The acceptable daily intakes of Cd, Cr, Fe, Mn, Pb and Zn were calculated on the basis that the adult person (70 kg weight) is consuming 100 g egg/day according to recommendations by AL-Ashmawy (2013). The results were compared with those recommended by FAO/WHO (1972, 1974c, 1982, 1987, 1989) (Table 3).

Table 3: Comparison of acceptable daily intake (ADI) value of heavy metals with the calculated daily intake from farm hen eggs and home-grown hen eggs

Metal	ADI mg*/ 70 kg person	Mean of metal in total examined farm hen egg samples (mg/kg)	Calculated average daily intake of metals from consuming of 100g egg/day (mg/d/person)	Mean of metal in total examined home-grown egg samples (mg/kg)	Calculated average daily intake of metals from consuming of 100 g egg/day (mg/d/person)
Cd	0.07	0.82	0.08	0.30	0.03
Fe	56	87.45	8.75	45.94	4.59
Mn	0.66	10.33	1.03	10.37	1.04
Pb	0.5	3.43	0.34	3.67	0.37
Zn	70.0	82.74	8.27	77.10	7.71

* According to FAO/WHO (1972, 1974c, 1982, 1987, 1989)

RESULTS AND DISCUSSION

Eggs, in particular those produced by hens on rural farms or for domestic use, are an important matrix for analysis with regard to both food safety, as they are a widely consumed foodstuff, and environmental control, in fact eggs from free-range hens are considered good indicators of the contamination of the environment in which the hens live (Van Overmeire*et al.*, 2006; Giannenas *et al.*, 2009). Analysis of hen egg samples in order to determine content of essential and toxic metals (Ca, Cd, Cr, Fe, Mg, Mn, Pb and Zn) were made. The results are shown in Table 4.

As regards toxic metals, either the European Union or the United States Food and Drug Administration do not establish limits on toxic metals in hen eggs. So, we compared our results with permissible limits of heavy metals in hen eggs set by Zmudzki and Szkoda (1996) and Roychowdhury *et al.* (2003). Permissible limits of heavy metals in hen eggs are as follows: Cd (0.05 mg/kg), Pb (0.5 mg/kg), Zn (20 mg/kg) (Zmudzki and Szkoda, 1996) and Cr (0.002 mg/kg) (Roychowdhury *et al.*, 2003).

Literature values of metal content from various parts of the world (total examined egg samples) and mean metal content obtained in this study are presented in Table 5.

Table 4: Obtained metal concentrations in hen eggs at different geographic areas in Bosnia and Herzegovina

Sample	Ca (mg/kg) ± SD	Cd (µg/kg) ± SD	Cr (mg/kg) ± SD	Fe (mg/kg) ± SD	Mg (mg/kg) ± SD	Mn (mg/kg) ± SD	Pb (mg/kg) ± SD	Zn (mg/kg) ± SD
1FA	794.57 ±93.72	ND	2.23 ±0.08	41.66 ±10.21	58.70 ±11.25	5.05 ±0.08	1.62 ±0.03	22.24 ±2.88
2FA	707.19 ±25.60	ND	1.89 ±0.56	56.43 ±7.03	11.70 ±2.10	5.42 ±0.14	1.62 ±0.02	30.55 ±8.94
3FA	381.84 ±39.35	ND	0.36 ±0.11	26.91 ±2.41	68.68 ±3.67	4.44 ±0.10	1.60 ±0.06	16.86 ±1.63
4FA	732.72 ±53.84	ND	ND	32.23 ±4.22	73.45 ±4.25	4.96 ±0.27	1.61 ±0.06	30.24 ±1.79
5FA	531.00 ±91.70	ND	ND	45.05 ±5.74	43.00 ±11.70	4.60 ±0.11	1.57 ±0.04	23.40 ±2.05
1HA	334.96 ±30.30	ND	ND	14.05 ±3.39	6.12 ±1.91	6.05 ±1.08	1.68 ±0.04	18.98 ±4.20
2HA	659.73 ±9.63	ND	ND	8.60 ±1.75	11.56 ±2.56	5.68 ±0.67	1.65 ±0.05	24.47 ±1.70
3HA	680.95 ±44.58	ND	ND	9.31 ±1.30	9.18 ±3.74	5.66 ±0.36	1.77 ±0.11	2.56 ±2.34
4HA	723.89 ±13.16	ND	ND	16.21 ±1.06	7.22 ±1.20	5.30 ±0.05	1.80 ±0.04	16.78 ±1.62
5HA	739.62 ±17.73	ND	ND	19.31 ±3.06	10.37 ±5.36	5.55 ±0.25	1.85 ±0.10	25.84 ±3.41
1FY	2982.02 ±410.21	0.61 ±0.26	ND	64.44 ±1.89	147.61 ±10.91	5.88 ±0.14	1.86 ±0.03	51.45 ±7.54
2FY	2128.86 ±418.95	0.64 ±0.23	ND	61.91 ±6.78	132.05 ±6.86	5.34 ±0.35	1.80 ±0.03	58.41 ±9.31
3FY	2354.15 ±188.26	1.23 ±0.17	ND	31.63 ±5.57	159.88 ±15.22	5.09 ±0.22	1.87 ±0.07	54.02 ±2.58

4FY	1603.87 ±71.96	0.97 ±0.06	ND	36.17 ±0.91	120.20 ±3.71	5.03 ±0.10	1.89 ±0.12	56.60 ±1.91
5FY	2325.45 ±16.92	0.67 ±0.22	ND	40.80 ±7.36	136.50 ±2.22	5.86 ±0.25	1.72 ±0.08	56.94 ±4.85
1HY	1385.02 ±103.34	0.20 ±0.02	ND	29.13 ±6.22	101.83 ±4.31	4.29 ±0.34	1.89 ±0.06	41.77 ±1.46
2HY	1313.51 ±77.09	0.22 ±0.05	ND	27.72 ±2.42	124.78 ±4.57	4.67 ±0.18	1.94 ±0.04	85.96 ±9.19
3HY	1309.71 ±133.31	0.25 ±0.02	ND	31.05 ±3.71	112.69 ±6.41	5.01 ±0.22	1.92 ±0.05	58.20 ±12.49
4HY	1313.16 ±111.56	0.24 ±0.06	ND	34.21 ±5.90	112.93 ±14.91	4.70 ±0.21	1.90 ±0.08	50.46 ±3.60
5HY	1302.94 ±97.58	0.57 ±0.04	ND	40.09 ±3.11	126.56 ±5.27	4.96 ±0.11	1.96 ±0.05	60.46 ±7.22

ND – not detected

Table 5: Literature values of metal content from various parts of the world (total examined egg samples) and mean metal content obtained in this study

Study	Ca	Cd	Cr	Fe	Mg	Mn	Pb	Zn
	(mg/kg)							
Bangladesh (Chowdhury *et al.*, 2011)	-	1.04	*<LOQ	124.58	221.29	-	12.1	104.14
Canary Islands (Rubio *et al.*, 2017)	853.3	-	0.14	20.06	112.9	0.36	0.06	15.82
Egypt (Hashish *et al.*, 2012)	2588	0.012	-	-	442.6	-	0.303	64.3
Germany (Souci *et al.*, 1999)	1510	-	-	72	280	0.5-2	-	40.2
Spain (Moreiras*et al.*, 2005)	1350	-	-	62	260	-	-	40
Turkey (Demirulus, 2013)	-	0.65	-	-	-	11.3	-	46.5
This study (Home-grown hen egg, mean value)	1953.70	0.30	**ND	45.94	124.65	10.37	3.67	77.10
This study (Farm hen egg, mean value)	2908.33	0.82	1.49	87.45	190.34	10.33	3.43	82.74

*LOQ – limit of quantification; ** ND – not detected

Calcium

Mean calcium concentration in the albumen of hen eggs taken from the farm was 629.46 mg/kg and 635.03 mg/kg of a home-grown hen egg albumen. Calcium concentrations in the farm eggs yolk were in the range from 1603.87 mg/kg to 2982.02 mg/kg. Small difference in calcium concentrations among samples of home-grown hen eggs yolk, ranging from 1302.94 mg/kg to 1385.02 mg/kg, was evidenced. The average concentration of this essential metal in home-grown eggs was 1324.84 mg/kg and 2278.87 mg/kg for farm eggs. The higher calcium concentration in eggs collected from farm may be due to the its higher content in the feed, so-called concentrate, since the higher calcium requirements for the female during egg production may be resulted in increased calcium absorption in the gut and consequently increased it content in eggs (AL-Ashmawy, 2013). Similar data were reported by Hashish *et al.* (2012), while lower concentrations were detected by Rubio *et al.*(2017), Souci *et al.*(1999) and Moreiras *et al.* (2005).

Magnesium

It was found that the magnesium content in the albumen from home-grown hen eggs was very low and ranged from 6.12 mg/kg to 11.56 mg/kg. In contrast to that, concentrations of this metal in samples of farm eggs were significantly higher, ranging from 11.70 mg/kg to 73.45 mg/kg. Also, magnesium concentrations were high in the farm eggs yolk (the highest concentration was 159.88 mg/kg and the lowest was 120.20 mg/kg), while the highest and lowest concentrations of magnesium for home-grown eggs yolks were 126.56 mg/kg and 101.83 mg/kg, respectively. Thus, unlike albumens, no significant difference was found in the magnesium content of egg yolks from domestic production compared to eggs from farm. Similar results for magnesium content in eggs were reported by Chowdhury *et al.*(2011) and Rubio *et al.*(2017) while higher concentrations were reported by Hashish *et al.*(2012), and slightly higher by Souci *et al.*(1999) and Moreiras*et al.*(2005).

Zinc

Mean concentration of zinc in the samples of HA was 21.33 mg/kg, whereas the mean value of this metal content in the FA was slightly higher, 24.66 mg/kg. The lowest zinc content for HY was 41.77 mg/kg, while the highest was 85.96 mg/kg, which was also the highest concentration of this metal for all examined samples. Among the FY, small difference in zinc levels were obtained, ranging from 51.45 mg/kg to 58.41 mg/kg. Similar data were reported forEgypt (Hashish *et al.*, 2012) while lower concentrations were determined in Germany, Spain,Turkey and Canary Islands(Souci *et al.*, 1999; Moreiras *et al.*, 2005; Demirulus, 2013; Rubio *et al.*, 2017) and higher concentration were detected in Bangladesh (Chowdhury *et al.*, 2011). Zn concentration levels in egg samples were above the permissible limits (20 mg/kg) set by Zmudzki and Szkoda (1996). Table 5 declares that average concentration of Zn in all the examined farm hen egg samples was 82.74 mg/kg that gives daily intake of about 8.27 mg/day/person from consumption of 100 g eggs that contributed about 11.81% of ADI recommended by FAO/WHO (1972, 1974c, 1982, 1987, 1989) and for home-grown hen eggs daily intake was 7.71 mg/day/person that contributed about 11.01% of recommended ADI.

Iron

Iron concentration in HA were in the range from 8.6 mg/kg to 19.31 mg/kg, which was lower than in the case of FA, from 26.91 mg/kg to 56.43 mg/kg. The highest content of iron in FY was 64.44 mg/kg while the lowest was 31.63 mg/kg. On the other hand, the difference in concentrations of this metal in the HY samples was slight lower ranging from 19.13 mg/kg to 40.09 mg/kg. Iron is essential trace element which required by humans nevertheless, but all metals are toxic at higher concentrations. The average of Fe concentration levels in eggs according to the USDA (2011) is 17.6 mg/kg. If the obtained results are compared to literature data about the metal content in eggs from other countries it can be noted that similar values are obtained in studies reported by Moreiras *et al.* (2005) and Souci *et al.* (1999). Average daily intake of iron from home-grown hen eggs from consuming of 100 g egg/day was 4.59 mg/d/person that contributed about 8.20% of ADI recommended by FAO/WHO (1972, 1974c, 1982, 1987, 1989) and daily intake of iron from farm hen eggs was 8.75 that contributed about 15.63% of recommended ADI.

Manganese

The contents of manganese in HA were lower but the within the approximate range (from 5.30 mg/kg to 6.05 mg/kg), while the ranging for FA was from 4.44 mg/kg to 5.42 mg/kg. Unlike the egg albumen's, higher content of manganese was obtained for farm yolk samples. Among these samples, the highest obtained concentration was 5.88 mg/kg and the lowest was 5.03 mg/kg, while the manganese concentration in HY ranged from 4.29 mg/kg to 4.96 mg/kg. Very similar results for manganese concentrations in eggs were reported by Demirulus (2013) while significantly lower concentrations were reported by Souci *et al.* (1999) andRubio *et al.* (2017). Average daily intake of manganese from farm hen eggs was 1.03 mg/d/person that contributed about 156.06% of ADI recommended by FAO/WHO (1972, 1974c, 1982, 1987, 1989) and intake of manganese from home-grown hen eggs was 1.04 that contributed about 157.58% of recommended ADI.

Cadmium

The concentrations of cadmium in all albumen samples were below the limit of detection of the used method (FAAS). Higher concentration of this metal (1.23 µg/kg) in comparison to other samples was obtained for sample 3FY, while the lowest concentration of cadmium was 0.61 µg/kg. On the other hand, cadmium contents in all HY samples were approximate and ranged from 0.20 µg/kg to 0.25 µg/kg. Similar results for cadmium in eggs were reported byDemirulus (2013) andChowdhury *et al.* (2011). Cd contents did not show increased level than the permissible limits of 0.05 mg/kg set by Zmudzki and Szkoda (1996), indicating that these foodstuffs are reasonably safe from Cd toxicity. Average daily intake of cadmium from farm hen eggs was 0.08 mg/d/person that contributed about 114.29% of ADI recommended by FAO/WHO (1972, 1974c, 1982, 1987, 1989) and intake of cadmium from home-grown hen eggs was 0.03 that contributed about 42.86% of recommended ADI.

Lead

Generally, higher lead concentration were obtained for HA (1.85 mg/kg) and HY (1.96 mg/kg) samples, while the lowest were for in the case of FA (1.62 mg/kg) and FY (1.72 mg/kg). If the obtained results are compared to literature data about the lead content in eggs from other countries it can be noted that lower values are obtained in studies reported by Hashish *et al.* (2012) and Rubio et *al.* (2017). Pb concentration levels in all egg samples were above the permissible limits (0.5 mg/kg) set by Zmudzki and Szkoda (1996). Average daily intake of lead from farm hen eggs was 0.34 mg/d/person that contributed about 68.00% of ADI recommended by FAO/WHO (1972, 1974c, 1982, 1987, 1989) and daily intake of lead from home-grown hen eggs was 0.37 that contributed about 74.00% of recommended ADI.

Chromium

The concentrations of chromium in all egg samples were below the limit of detection of the used method (FAAS), except in the case of albumen of eggs from farms where the concentration ranged from ND to 2.23 mg/kg. Detected Cr concentrations were above the permissible limits (0.002 mg/kg) set by Roychowdhury*et al.* (2003).

The concentration of metals varied between different samples. By concentrations the metals were arranged as the following diminishing series: (a) home-grown hen egg albumen: Ca > Zn > Fe > Mg >Mn>Pb; (b) home-grown hen egg yolk: Ca > Mg > Zn > Fe >Mn>Pb> Cd > Cr; (c) farm egg albumen: Ca >Mg >Fe > Zn >Mn>Pb> Cr > Cd; (d) farm egg yolk: Ca > Mg > Zn > Fe >Mn>Pb> Cd > Cr.

CONCLUSION

Results of the present study revealed that content of all examined essential metals was higher in yolks compared to albumens, both farm and home-grown hen eggs. The content of zinc in all samples was higher than the permissible limit value. The most significant difference in the concentrations was obtained for iron in albumens. Deviations in the concentrations of the examined metals have been obtained for farm poultry egg samples (FA and FY), while the concentrations of certain metals in samples of eggs from domestic breeding hens were balanced with smaller discrepancies. Minimum difference in concentrations of examined metals among the farm and home samples was obtained for manganese. Among the toxic metals, chromium was present only in a few FA samples, while its concentrations in the HA samples, as well as in all yolk samples were below the detection limit of the used method (FAAS). Results obtained for cadmium have shown that this metal was below the LOD in albumen samples, and in yolk samples low concentrations were detected. The highest (Fe) and lowest (Cd) average daily intake from consuming of 100g egg/day were obtained for farm hen eggs. Average daily intake of Zn and Cd from consuming of 100g egg/day from home-grown hen eggs had the highest and the lowest value, respectively. Eggs are a notable essential elements source and the consumption of eggs does not necessarily mean a considerable contribution of toxic metals.

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