

DETERMINATION OF 17 ELEMENTS IN FREE-RANGE HEN EGGS WITH ICP-MS

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Abstract. The egg is a widely consumed food material with the higher nutritional value obtained from poultry. It contains almost all the nutrients needed by metabolism, therefore, the biological value of an egg is high. The aim of this study is to determine the levels of eighteen elements in free-range hen eggs. Eggs (n=217) were collected from February 2017 to September 2017 in the spring, summer and autumn seasons from towns near the Syrian border. The ICP-MS method was used to determine the Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Ti, As, Cd, Tl and Pb elements levels in the study. The most abundant elements were Na and K ranging between 248.12-7755 mg/kg and 80.25-4235 mg/kg, respectively. When the element levels of the free-range hen egg samples were examined, it was seen that there was a statistical difference between the seasons while the samples were contaminated with As on an average of 1131.25 µg/kg when the toxic element levels were examined. It was concluded that the element content of free-range hen eggs produced in this region was affected by seasonal changes.

Keywords: *free-range hen eggs, ICP-MS, macro element, micro element, toxic element*

Introduction

Occupation and income status of a society influences nutritional habits and thereby human health. Due to easily accessible, the widely available food source of hen eggs, which are basic food ingredients, consumed a lot in the worldwide. Hen egg has important nutritional value and contributes significantly to a healthy diet with its' high-quality proteins, vitamins, fats, and minerals (Domingo, 2014). A medium-sized egg includes 78 kcal, consisting of 6.5 g of protein, 2.3 g monounsaturated fat on an average. Most of the lipids of the egg are in the form of lipoproteins found in egg yolk as well as proteins located in all parts of the egg (Kovacs-Nolan et al., 2005; Domingo, 2014). It also includes dietary cholesterol, unsaturated fatty acids, and minerals (Fernandez, 2012). In continents surveyed by Food and Agriculture Organization of the United Nations, per capita egg consumption in 2009 varied from 2.3 kg in Africa to 12.7 kg in Europe (FAO, 2012). According to the Central Association of Egg Producers of Turkey (YUM-BIR), Turks consume 200 chicken eggs per person per year (YUM-BIR, 2015).

In recent years consumers began to prefer free-range hen eggs although the yolk color of free-range eggs was lower than that of battery-reared hen eggs (Kucukkoyuncu et al., 2017). Instead of battery-reared system eggs owing to the common wisdom that free-range hen eggs have preferable nutritional quality. In the free-range system, hens

have opportunity pasture foraging in around. A close relationship with the environment may cause to be exposed to contaminants (Van Overmeire et al., 2006). And even free-range hen eggs are expected good bio-indicators of the contaminants of the environment in where the hen's pasture (Chang et al., 1989). Despite this well-known view, home-produced foods like free-range hen eggs are not analyzed to any convenience check by a routine program. Monitoring the levels of the macro, trace, and potentially toxic elements are one of the aspects of the food and also environmental quality. Macro and trace elements are found naturally and essential for throughout life (Esposito et al., 2016). But toxic elements such as Pb, Cd, As known to be toxic and have maximum limits by European Commission in some foods but not for eggs (European Commission Regulation, EC No 1881, 2006). The European Food Safety Authority has reported that the environmental pollutants such as toxic element detected in free-range hen eggs that pasture outdoors and more exposed to environmental contamination were higher than those detected in battery-reared hen eggs (EFSA Scientific Report—update of the monitoring of levels of dioxins and PCBs in food and feed, 2012). Actually, a close relationship with the soil identifies higher levels of Co, Zn, Pb in free-range hen eggs (de Freitas et al., 2013).

Şanlıurfa is located along the Syrian border and has four districts on the borderline. These are; Akçakale, Birecik, Harran, and Suruç. In these districts, that they are close of the Syrian war which is ongoing for a long time, the possible transition of toxic element contaminants from the environment to the local consumers and even to the food chain are not existing. The design of this paper focuses on the content of thirteen essential elements (Na, Mg, K, Ca, Cr, Mn, Fe, Co, Cu, Zn, Se, Ni, Ti) and four toxic elements (As, Pb, Tl, Cd) in fresh eggs of free-range hen eggs which should be bio-indicator of pollution. To the best of our knowledge, this study provides the first seasonal variation of elements in free-range hen eggs worldwide. The aims were for (1) an evaluation of concentrations of elements, (2) a vision of seasonal variations, and (3) indication of cautious risk affects.

Material and Methods

Samples

A total of 217 fresh eggs from free ranged hens were used as study material. The samples were separated depending on the seasonal variations as

- i) 60 eggs collected in the spring season.
- ii) 73 eggs collected in the summer season.
- iii) 84 eggs collected in the autumn season.

The samples were collected from home producers from Akçakale, Birecik, Harran, Suruç between March to October 2017 as shown in *Figure 1*. They were transported to the laboratory and stored at 4°C until analyses.

Sample preparation and analytical procedures

Egg samples were homogenized by taking into 50 ml sterile falcon tubes. Whole equipment was previously kept for one night in 10% HNO₃ and then washed with ultrapure water to eliminate possible contamination of elements. One g of the homogenized egg samples were mineralized acid digestion with 6 ml HNO₃ 65% (v/v) (Merck, Germany) and 2 ml H₂O₂ 30% (v/v) (Merck, Germany) in PTFE vessels of

Mars Xpress (CEM Corporation) microwave system as described in the literature (Esposito et al., 2016). Subsamples were treated as the following procedure; up to 120°C for 7 min 1600 W and constant 5 min, up to 160°C for 5 min 1600 W and constant 5 min, up to 210°C for 20 min 1600 W and constant 5 min. The same procedure was done for every blank sample (1 ml ultrapure water). After the cooling stage to room temperature (22-23°C), the subsamples were diluted to 50 ml with ultrapure water (MES MP Mini pure, Turkey).

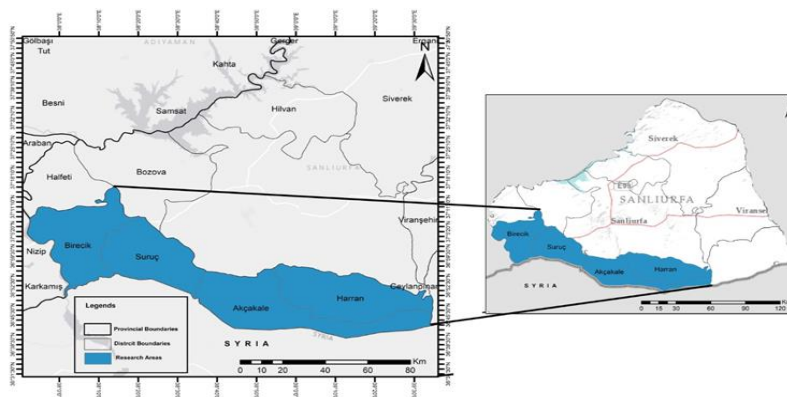


Figure 1. Map of the study area

Elemental analyses were done by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7500ce with an Octopole Reaction System) equipped with a nebulizer (Agilent, Japan), autosampler (Cetac ASX-520), and spray chamber.

All analytical methods were performed according to the method of UNI EN 15763: 2010 preferably modified. The following isotopes were measured: $^{23}\text{Na}^+$, $^{24}\text{Mg}^+$, $^{39}\text{K}^+$, $^{44}\text{Ca}^+$, $^{52}\text{Cr}^+$, $^{55}\text{Mn}^+$, $^{56}\text{Fe}^+$, $^{59}\text{Co}^+$, $^{63}\text{Cu}^+$, $^{66}\text{Zn}^+$, $^{78}\text{Se}^+$, $^{75}\text{As}^+$, $^{206}\text{Pb}^+$, $^{60}\text{Ni}^+$, $^{48}\text{Ti}^+$, $^{205}\text{Tl}^+$, $^{111}\text{Cd}^+$.

Quality controls were assured to confirm the accuracy of the analytical procedure with the reference materials (NIST SRM 1515 Apple Leaves). The limit of detection and quantification were calculated as three and ten times.

Analyses of certified reference material allowed an assessment of accuracy and precision over a range of element concentrations. Certified values indicated that monitored levels correlated well with certified levels as shown in *Table 1*.

Statistical Analysis

For testing the normality of the data, the Shapiro-Wilk test was performed. In order to compare normal non-dispersive variables in more than two independent groups, Kruskal Wallis and All Pairwise Multiple Comparison tests were used. Spearman Rank Correlation Coefficient was used in testing the relations between numerical variables. For descriptive statistics, mean \pm standard deviation values for numerical variables are given. Values of $P < 0.05$ was considered as statistically significant. All statistical analyses were performed in order to verify the existence or not of significant differences between the samples. SPSS for Windows version 24.0 package program (SPSS Inc., Chicago, USA) was used for statistical analysis. When the concentration of the element was below the limit of quantification (LOQ) that value did not participate in the statistic.

Table 1. Element concentrations in certified reference material (NIST SRM 1515 Apple Leaves)

Element	Certified data (mg/kg)	Our data (mg/kg)	Recovery (%)
Na	10.0	9.96	99.6
Mg	10.0	9.95	99.5
K	10.0	9.98	99.8
Ca	10.0	10.2	102
Cr	10.0	9.95	99.5
Mn	10.0	9.90	99.0
Fe	10.0	10.4	104
Co	10.0	9.93	99.3
Cu	10.0	9.99	99.9
Zn	10.0	9.97	99.7
Se	10.0	9.96	99.6
Ti	10.0	10.1	101
As	10.0	9.97	99.7
Pb	10.0	10.3	103
Ni	10.0	10.4	104
Tl	10.0	9.95	99.5
Cd	10.0	10.5	105

Results and Discussion

The concentration of Sodium, Magnesium, Potassium, Calcium, Chromium, Manganese, Iron, Cobalt, Copper, Zinc, Selenium, Arsenic, Lead, Nickel, Titanium, Talium, Cadmium in the egg samples are listed in *Table 2*, which reports mean and standard deviation in different seasons and *Table 3* reports median and quartiles of free-range hen eggs. Box plot analyses of groups of data was shown in *Figure 2*.

Table 2. Comparison of concentrations of elements between seasons

Element	Positive sample (n)	Spring (Mean ± SD)	Positive sample (n)	Summer (Mean ± SD)	Positive sample (n)	Autumn (Mean ± SD)
Na (mg/kg)	60	1278.08 ±324.27	73	4329.86 ±1460.72	84	1707.73 ±384.32
Mg (mg/kg)	60	246.15 ±31.44	73	143.54 ±25.57	84	157.76 ±17.47
K (mg/kg)	60	1035.92 ±342.37	73	1953.67 ±775.17	84	1964.9 ±437.86
Ca (mg/kg)	60	1067.33 ±219.75	73	490.53 ±167.6	84	560.54 ±111.96
Cr (µg/kg)	13	543.92 ±35.38		<LOQ		<LOQ
Mn (µg/kg)	34	709.41 ±152.94		<LOQ	6	576.16 ±35.38
Fe (mg/kg)	60	39.9 ±12.79	73	112.01±25.76	84	45.65 ±8.88
Co (µg/kg)		<LOQ		<LOQ		<LOQ
Cu (µg/kg)	14	974.12 ±850.27	73	9904.66 ±4004.7	84	2012.95 ±365.29
Zn (mg/kg)	60	32.34 ±7.44	60	11.80 ±6.50	84	22.77 ±4.59
Se (µg/kg)	60	7354.92 ±2972.93	38	9875.39 ±4522.55	84	11834.66 ±3624.58
Ti (µg/kg)	60	3940.1 ±578.65	35	3021.57 ±353.47	84	2125.68 ±247.91
As (µg/kg)	3	841.66 ±263.45	1	2545	4	990.25 ±70.35
Pb (µg/kg)		<LOQ		<LOQ		<LOQ
Ni (µg/kg)	20	140.25 ±109.45		<LOQ		<LOQ
Tl (µg/kg)		<LOQ		<LOQ		<LOQ
Cd (µg/kg)		<LOQ		<LOQ		<LOQ

Table 3. Values of median, 25th, and 75th variance for essential elements in free-range hen eggs

	Spring			Summer			Autumn		
	Quartile 1	Median	Quartile 3	Quartile 1	Median	Quartile 3	Quartile 1	Median	Quartile 3
Na(mg/kg)	1068.76	1234.68	1417.49	3202.50	4137.50	5655.00	1510.40	1763.60	1954.40
Mg(mg/kg)	223.07	241.39	262.26	126.35	144.35	158.80	147.33	157.53	166.73
K (mg/kg)	846.30	997.43	1179.55	1549.00	1848.00	2502.50	1831.00	2020.00	2177.50
Ca(mg/kg)	878.75	1095.00	1217.25	360.25	489.25	611.50	522.00	558.00	601.00
Fe (mg/kg)	32.89	39.12	46.82	93.15	104.68	121.53	39.74	45.32	50.10
Cu (µg/kg)	137.50	824.05	1895.85	7290.00	9027.50	13525.00	1796.00	2026.25	2251.50
Zn(mg/kg)	26.80	33.01	36.86	6.55	11.19	15.03	20.62	22.447.50	25.45
Se (µg/kg)	5048.30	6522.85	8820.85	6510.00	8668.75	12702.50	9935.00	11505.00	14555.00

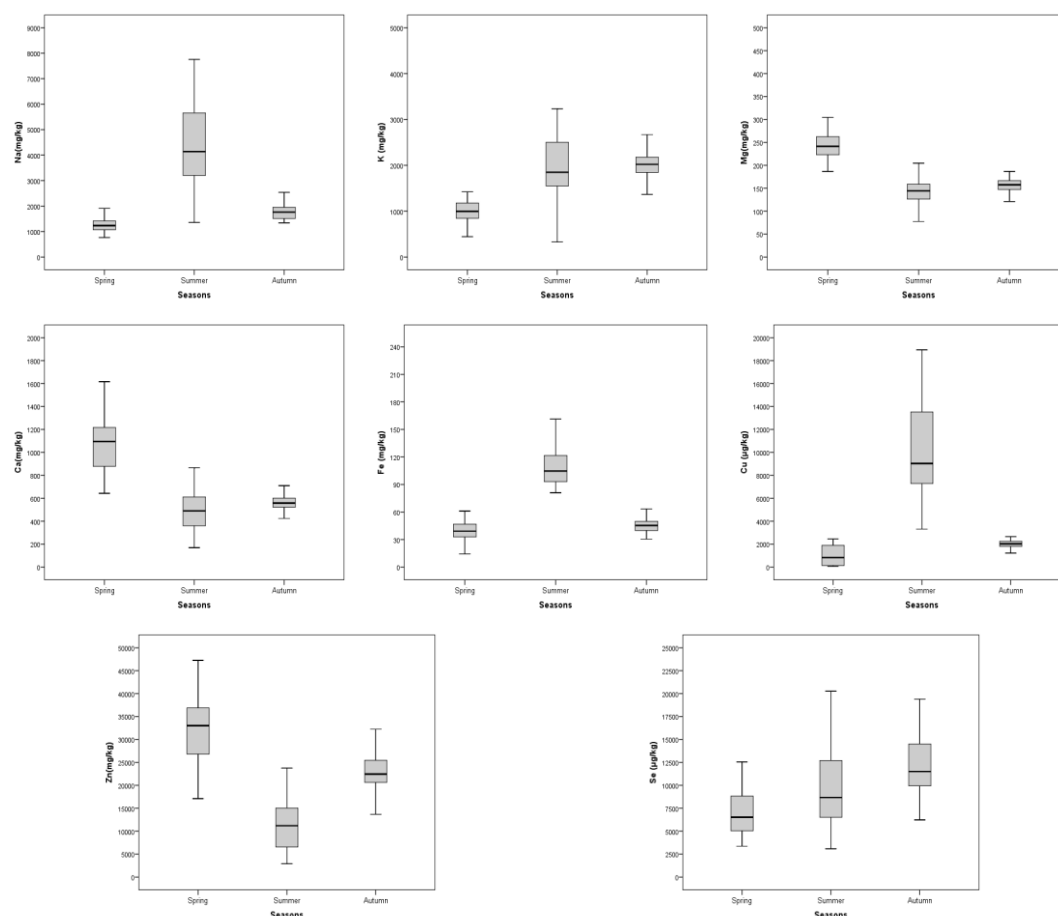


Figure 2. Box plot of essential elements in free-range hen eggs

In the present study the sodium content of free-range hen eggs showed an average level of 1278.08 mg/kg in the spring season; this is relatively low when compared with the mean level (1707.73 mg/kg) in the autumn season and also very low when compared with the mean level (4329.86 mg/kg) in the summer season. *Table 4* shows the

comparison of the levels of elements reported in the different samples of home-grown hen and free-range hen eggs with previous literature. In the literature, the sodium content of eggs showed a mean level of 1149 mg/kg; this is lowly especially compared with the present study (Rubio et al., 2017).

Magnesium concentration of spring, summer, and autumn seasons in free-range hen eggs showed mean levels of 246.15, 143.54, 157.76 mg/kg, respectively. In the literature, a lower value have been reported in Canary Islands (87.5 mg/kg), while value as high as has been found in home-produced eggs from Brazil (674 mg/kg) (de Freitas et al., 2013).

The average levels of potassium detected in free-range hen eggs were 1034.92 mg/kg in spring, 1953.67 mg/kg in summer, and 1964.9 mg/kg in the autumn season. These values are high, especially when compared with the reported data in the literature (Rubio et al., 2017, 2018).

These latter tables report calcium concentrations of 1067.33 mg/kg in spring season while the value is higher than both summer (490.53 mg/kg) and autumn (560.54 mg/kg) seasons. The concentrations in our samples were also comparable to that reported in free-range hens egg from the Island of Tenerife with a mean level of 231 mg/kg (Rubio et al., 2018).

The average level of chromium in the spring season (543.92 µg/kg) is much higher than that detected in both summer (<LOQ) and autumn (<LOQ) season. In the spring, due to the fact that the chromium element is detected in egg samples, agricultural activities in the region are intense and pesticides and foliar fertilizers are generally applied in spring.

The manganese is one of the most abundant elements in the earth crust. The manganese mean content in the spring season (709.41 µg/kg) was higher than the mean content in autumn season (576.16 µg/kg) and in free-range hen eggs in United Kingdom (0.277 mg/kg) (Siddiqui et al., 2011). The iron mean content in the spring season (39.9 mg/kg) was lower than the values found in summer season (112.01 mg/kg) but similar to that analyzed in autumn season (45.65 mg/kg). The mean concentration of iron determined in free-range hen eggs proved to be far higher than the levels reported in eggs in the Island of Tenerife (7.62 mg/kg) (Rubio et al., 2018) and parallel with eggs in Brazil (114 µg/g) (de Freitas et al., 2013).

In the present study, the average level of copper was significantly increased from 974.12 to 9904.66 µg/kg between spring season to summer season afterward in the autumn season the mean copper content was 2012.95 µg/kg. The change rule of the concentration of copper was similar to iron.

The average concentration of Zinc in free-range hen eggs in spring season (32.34 mg/kg) was significantly higher than both summer (11.80 mg/kg) and autumn (22.77 mg/kg) season and was 10-fold higher than reported in fresh cage-reared hens eggs from Canary Islands (Rubio et al., 2017).

The average level of selenium in free-range hens eggs was increased from 7354.92 to 11834.66 between spring to autumn season and marginally higher than found in eggs from residential backyards in Italy (Esposito et al., 2016).

Arsenic is a micropollutant which can be easily detected in the soil, water, fertilizer, and industrial wastes. Long term exposures, arsenic is accumulated in metabolism and should because chronic diseases (Hashemi et al., 2018). The mean concentration of arsenic in free-range hen eggs was 841.66 µg/kg in the spring season, 2545 µg/kg in the summer season, and 990.25 µg/kg in the autumn season.

Table 4. Comparison of the levels of elements reported in the different samples of home-grown hen and free-range hen eggs

Element	Rubio et al. (2017)	Rubio et al. (2018)	Esposito et al. (2016)	Grace and MacFarlane (2016)	De Freitas et al. (2013)	Van Overmeire et al. (2006)	Fakayode and Olu-Owolabi (2003)	Hashemi et al. (2018)	This study Spring	This study Summer	This study Autumn
Na (mg/kg)	1149±96.2	1290±154							1278.08 ± 324.27	4329.86±1460.72	1707.73±384.32
Mg (mg/kg)	87.5±26.1	103±13			674±138				246.15±31.44	143.54 ± 25.57	157.76±17.47
K (mg/kg)	491±217	712±135							1035.92±342.37	1953.67±775.17	1964.9±437.86
Ca (mg/kg)	174±83.3	231±56							1067.33±219.75	490.53±167.6	560.54±111.96
Cr (µg/kg)	170±240	50.0±90.0	8.0±9.0					1621	543.92±35.38	<LOQ	<LOQ
Mn (µg/kg)	100±110	140±70	360±142		1500±600	313.98			709.41±152.94	<LOQ	576.16±35.38
Fe (mg/kg)	5.49±4.10	7.62±2.7			114±30		23.20		39.9±12.79	112.01±25.76	45.65±8.88
Co (µg/kg)	<LOQ	<LOQ	4.0±2.0		19100±12500	5.15	10		<LOQ	<LOQ	<LOQ
Cu (µg/kg)	2220±1100	1780±780	679±128	750	2900±500	603.64	780		974.12±850.27	9904.66±4004.7	2012.95±365.29
Zn (mg/kg)	3.37±1.73	5.03±1.4	15.75±4.05	11.3	65.3±20.3	11.543	13.75		32.34±7.44	11.80±6.50	22.77±4.59
Se (µg/kg)			313±147		600±200	272.97			7354.92±2972.93	9875.3±4522.5	11834.6±3624.5
Ti (µg/kg)									3940.1±578.65	3021.5±353.4	2125.6±247.9
As (µg/kg)			7.0±4.0	30	21.5±8.5	15.95			841.66±263.45	2545	990.2±70.3
Pb (µg/kg)	20±20	30±20	19±28	90	89±245	68.56	590		<LOQ	<LOQ	<LOQ
Ni (µg/kg)	50±30	30±20	96±97			36.58	30		140.25 ± 109.45	<LOQ	<LOQ
Tl (µg/kg)			10±22			0.69			<LOQ	<LOQ	<LOQ
Cd (µg/kg)	<LOQ	<LOQ	3±3	1	0.6±0.5	0.53	70	13	<LOQ	<LOQ	<LOQ

The reported concentrations of arsenic in egg samples from Italy (Esposito et al., 2016), Australia (Grace and MacFarlane, 2016), Brazil (de Freitas et al., 2013) were 7.0, 30, and 21.5 µg/kg, respectively. The finding of this study is very high than the reported concentrations of arsenic in egg samples from different countries.

The mean value of nickel from Canary Island (Rubio et al., 2018), Italy (Esposito et al., 2016), and Turkey (Uluözlu et al., 2009) were 30, 96, and 90 µg/kg, respectively. These levels are lower than our study (140.25 µg/kg).

Element content in the free-range hen eggs in the spring season

The mean concentration and standard deviation of each element analyzed in the spring season are shown in *Table 5*. Sodium (1278.08 mg/kg) was the most abundant macro-element analyzed in the highest proportion in free-range hen egg samples, followed by Ca>K>Mg.

Table 5. Descriptive statistics of essential elements for the spring season

Elements	Akçakale	Birecik	Harran	Suruç
Na (mg/kg)	1280.7±152.97	1440.38±435.26	1244.67±266.59	1146.58±333.55
Mg (mg/kg)	236.3±25.72	254.7±33.09	248.43±39.06	245.17±26.33
K (mg/kg)	1073.22±167.25	1378.47±419.25	936.3±137.13	755.68±222.36
Ca (mg/kg)	979.97±211.01	1029.67±191.62	1074.1±210.94	1185.6±230.29
Fe (mg/kg)	39.27±11.72	41.86±9.36	41.22±18.52	37.26±10.37
Cu (µg/kg)	104.73±46.35	929.56±796.22	719.11±512.22	1729.1±1058.9
Zn (mg/kg)	28.90±6.85	31.06±5.28	32.18±7.15	37.21±8.22
Se (µg/kg)	7426.73±1208.47	11655.05±1955.3	5761.96±743.91	4575.93±774.25

The major trace element found in egg samples was selenium (7354.92 µg/kg), followed by Cu>Mn>Ti>Cr>Ni>Fe>Zn>Co. Arsenic (841.66 µg/kg) was the most abundant toxic element found in three free-range hen eggs; Pb, Tl, Cd levels were below the limit of quantification in all samples in the spring season.

Element content in the free-range hen eggs in the summer season

The mean concentration and standard deviation of each element analyzed in the summer season are shown in *Table 6*. The most abundant macroelement in the free-range hen eggs was sodium (4329.86 mg/kg), followed by K>Ca>Mg. As for the trace elements, copper (9904.66 µg/kg) was the most notable in the egg samples, followed by Se>Ti>Fe>Zn. The Cr, Mn, Co and Ni levels were below the limit of quantification in all samples in the summer season. The detected levels of Co and Cd were below the quantification limit. Arsenic (2545 µg/kg) was the most abundant toxic element found in 1 free-range hen egg; Pb, Tl, Cd levels were below the limit of quantification in all samples in the summer season.

Element content in the free-range hen eggs in the autumn season

The mean concentration and standard deviation of each element analyzed in autumn season are shown in *Table 7*. Potassium (1964.90 mg/kg) was the most abundant macro-element analyzed in the highest proportion in egg samples, followed by Na>Ca>Mg. The major trace element found in egg samples was selenium (11834.66 µg/kg),

followed by $Ti > Cu > Mn > Fe > Zn$. Co and Ni levels were below the limit of quantification in all samples in the autumn season. Arsenic (990.25 $\mu\text{g}/\text{kg}$) was the most abundant toxic element found in 4 free-range hen egg. The detected levels of Pb, Tl and Cd were below the quantification limit in the autumn season.

Table 6. Descriptive statistics of essential elements for the summer season

Elements	Akçakale	Birecik	Harran	Suruç
Na (mg/kg)	4195.5±969.41	5775.25±352.97	4142.97±370.31	2598.79±685.53
Mg (mg/kg)	155.15±19.87	141.31±24.37	136.66±15.39	131.76±34.43
K (mg/kg)	2006.99±385.34	2707.8±562.41	1764.94±229.93	1071.89±579.56
Ca (mg/kg)	570.69±177.35	463.68±111.54	421.13±167.73	450.91±143.39
Fe (mg/kg)	119.27±28.21	129.81±20.66	94.94±8.54	100.51±18.19
Cu ($\mu\text{g}/\text{kg}$)	9568.25±2581.11	14020.5±872.72	9079.53±705.53	5192.65±1501.68
Zn (mg/kg)	13.76±4.74	8.22±3.65	10.25±6.34	10.38±5.373.66
Se ($\mu\text{g}/\text{kg}$)	11117.12±4557.47	5320±2857.78	9170.77±2760.29	7160±0

Table 7. Descriptive statistics of essential elements for the autumn season

Element	Akçakale	Birecik	Harran	Suruç
Na (mg/kg)	1727.77±463.76	1965.72±174.47	1602.34±190.34	1378.14±443.87
Mg (mg/kg)	163.11±15.74	159.19±15.69	153.88±21.54	155.26±16.49
K (mg/kg)	1965.73±467.98	2129.5±239.09	2058±301.55	1586.44±579.32
Ca (mg/kg)	582.32±74.77	534.16±73.04	584.38±133.5	561.13±157.71
Fe (mg/kg)	46.45±9.19	41.97±5.34	50.88±8.71	45.33±11
Cu ($\mu\text{g}/\text{kg}$)	2189.9±267.48	2248.8±240.94	1880.1±235.71	1596.88±319.51
Zn (mg/kg)	23.10±3.385	21.50±3.47	23.90±7.02	23.44±3.13
Se ($\mu\text{g}/\text{kg}$)	8765.17±2472.89	10570.33±1635.91	14309.25±2485.36	13750.22±4963.94

Correlation between elements

Correlation between elements in free-range hen eggs samples was shown in *Table 8* for the spring season, *Table 9* for the summer season, and *Table 10* for the autumn season.

Table 8. The correlations between the of essential elements in the spring season

	Mg	K	Ca	Ti	Fe	Cu	Zn	Se
Na	.236	.584**	-.608**	-.580**	-.291*	.262	-.684**	.397**
Mg	1.000	.087	.136	.206	.227	.305	.066	.066
K		1.000	-.338**	-.366**	.076	.196	-.427**	.732**
Ca			1.000	.986**	.671**	-.196	.919**	-.366**
Ti				1.000	.644**	-.103	.903**	-.388**
Fe					1.000	.134	.644**	.001
Cu						1.000	-.066	-.240
Zn							1.000	-.428**

Notes: Spearman correlation test, * $P < 0.05$, ** $P < 0.01$

Table 9. The correlations between the essential elements in the summer season

	Mg	K	Ca	Ti	Fe	Cu	Zn	Se
Na	0.157	.907**	0.061	0.014	.470**	.964**	0.028	-0.287
Mg	1	0.197	.525**	.376*	.346**	0.163	.447**	0.242
K		1	0.227	-0.054	.626**	.906**	0.099	-0.204
Ca			1	.673**	.562**	0.171	.912**	0.073
Ti				1	0.304	0.027	.621**	0.403
Fe					1	.533**	.392**	0.193
Cu						1	0.138	-0.308
Zn							1	-0.115

Notes: Spearman correlation test, *P<0.05, **P<0.01

Table 10. The correlations between the elements in autumn season

	Mg	K	Ca	Ti	Fe	Cu	Zn	Se
Na	.379**	.538**	-.355**	.036	-.180	.642**	-.379**	-.394**
Mg	1.000	.235*	.006	.219	.139	.230*	-.007	-.196
K		1.000	-.131	.153	.093	.532**	.004	-.179
Ca			1.000	.804**	.502**	-.084	.819**	.116
Ti				1.000	.416**	.319**	.679**	-.224*
Fe					1.000	.013	.431**	.058
Cu						1.000	.047	-.624**
Zn							1.000	.105

Notes: Spearman correlation test, *P<0.05, **P<0.01

In spring season, very strong correlations were detected between CaxTi, CaxZn, TixZn, and also strong correlations were detected between NaxZn, NaxCa, KxSe, CaxFe, TixFe, FexZn as shown in *Table 7*. Interestingly there wasn't a correlation between elements with Mg, so further studies required.

In the summer season, very strong correlations were detected between NaxK, NaxCu, CaxZn, KxCu. Strong correlations were found between KxFe, CaxTi, TixZn as shown in *Table 9*. In autumn season, very strong correlations were detected between CaxTi, CaxZn, and strong correlation between NaxCu, TixZn, CuxSe as shown in *Table 10*. A correlation between CaxZn were detected in all seasons thus it means that the season is not influencing this very strong correlation.

Conclusion

In this study, the element levels of eighteen elements in the free-range hen egg samples were examined by means of ICP-MS. Free-range hen eggs are an acceptable source of essential elements, in particular, of Na, Mg, K, Ca, Cu, Fe, Zn, and Se. The presence of Cr in the spring season, Cu in the summer season and Se in all seasons in our samples may be related to a geographic source. Finally, the lack of data on the presence of some Ti in free-range hen eggs does not allow comparisons. It was seen that there was a statistical difference between the seasons so it was concluded that the

element content of free-range hen eggs produced in this region was affected by seasonal changes.

Compilation of these data may serve to understand the element composition and to define the reference levels of macro, trace and toxic elements to be used in food safety assessments of free-range hen eggs produced in an area near the Syrian border because it was determined that eight samples of free-range hen eggs were contaminated with As on an average of 1131.25 µg/kg when the toxic element levels were examined. It is important to regularly monitoring the levels of toxic elements in free-range hen eggs for assessing the risk of long-term exposure.

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