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**Japanese Journal of Veterinary Research**
Toxic metals in organic, home and commercially produced eggs, comparative and risk assessment study

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Abstract
Due to the great consumers’ demand for organic eggs as a safe alternative, this study was conducted to probe the chemical safety of Egyptian organic eggs and other regularly produced types, in term of toxic metals containment. Cadmium (Cd), Lead (Pb) and Aluminum (Al) were determined by atomic absorption spectroscopy in 150 eggs samples of organic, cage-free home and caged-commercially produced types. Obtained results seemed alarming for all types of tested eggs. 34% and 40% of organic eggs samples exceeded the maximum permissible limit of Cd and Pb, respectively. As a risk assessment model, Target Hazard Quotients (THQ) of examined eggs types presumed low health risk potential. However, Egyptian organic eggs did not seem to be more safer as had been promoted. This study urges reviewing of the regulatory legislations regarding organic food chemical safety.

Keywords: Toxic metals, organic eggs, lead, cadmium, target hazard quotients.

Introduction
Despite the huge diversity of available natural foods, few are being considered to solely have an admirable nutritional value. Among these foods, eggs are prominent type that supply human with many essential bioavailable nutrients. Eggs and their products consumption has steadily increased worldwide. Consequently, the necessity for assuring these products safety is of critical relevance to guard consumers’ health.
Different pollutants, mainly chemicals, may find their way into eggs and thus representing imminent health hazards. Among chemical pollutants, metals are ubiquitous due to both natural and artificial causes. However, not all metals are undesirable, as many metals are required for normal catabolic body processes. Metals find their way into eggs via hens’ feeds. In addition, hens exposure to environmental residues of these metals represents another important source. Owing to the tremendous increase in eggs direct consumption or use in the food industry, eggs are considered one of the most suspicious foods as a source of heavy metals human exposure. Recently, consumers’ preferences were greatly shifted toward products which are promoted as healthier choices. Organic eggs consumption, as one of these new preferences, is growing rapidly. Regulatory authorities have listed many conditions in order to grant an organic certification to eggs farms. Hens should get an organic feed, have a free access to land, do not get any antibiotics, steroids, vaccines or hormones, and the grains, which are...
included in the hens’ feed, must be harvested from land with no previous three-years exposure to any fertilizers or pesticides\(^{16}\). However, these regulations seem to be not sufficient to guarantee the concordance of organic eggs to the safe limit containment of toxic metals. In consideration of the above, this study was designed to investigate the quality of marketed eggs (Organic, cage-free home produced and caged commercially produced) in regards to the concentrations of some toxic metals, and to verify the meritoriousness of organic eggs as safer alternative to other regularly produced types.

**Materials and Methods**

*Samples collection and hens’ husbandry:* 150 random eggs samples [50 each of organic (OE), cage-free home (CFE) and caged-commercially produced (CCE) eggs] were collected from different outlets in their original packages through March, April, and July 2015. Organic eggs are enclosed in stamped packages which stated that the producer has been granted an organic eggs production’s certificate. Different organic eggs brands were chosen and only two eggs were chosen from each package. Each sample was labelled to identify the source, site, and date of sampling. CFE were produced from hens that kept at farmers’ homes and mostly fed on home leftover foods. CCE Producing hens were not allowed any access to outdoor land and received commercial feed only.

*Samples preparation:* Each egg was opened through the air cell using scissors and the entire content of each egg was placed in a clean dry glass dish, weighed and thoroughly homogenized. Contents were dried in hot air oven at 70°C. One gm of each dried sample was transferred into overnightly acid washed (Nitric acid, 65% Sigma) screw capped digestion tube. Digestion tubes were microwaved after addition of 6 mL of 65% Nitric acid and 6 mL of Milli-Q water (Millipore).

*Metals determination and calculation:* Following digestion, solutions were filtered and subjected to analysis using Atomic absorption spectroscopy (Buck scientific 210VGP Atomic Absorption Spectrophotometer) with different furnace source. An acetylene-air furnace was used to determine Pb and Cd, while Graphite furnace was used for Al. Analytical standardization was done using blank and metal standard solutions (Merck) and detection percentage were calculated to be at least 98%. Limit of detection (LOD) of each metal was tabulated in table (1). Metal concentration in eggs samples was calculated after subtraction of blank values as follow:

\[
\text{Metal concentration (ppm)} = r \times d \div w
\]

Where r is average of three instrumental reading for each metal, d is sample dilution and w is sample weight.

*Potential health risks Assessment:* For estimation of potential health hazards related to targeted toxic metals due to eggs consumption, Target Hazard Quotients (THQ) was calculated as follow:

\[
\text{THQ} = \frac{EF \times ED \times FIR \times C}{RfD \times W \times T} \div 1000
\]

where EF is exposure frequency (number of days of exposure per year), ED is the exposure duration (average lifetime which is assumed to be 70 years\(^{8}\)), FIR is food ingestion rate (the ingested amount in grams of eggs in a day), C is metal concentration in examined eggs, RfD is the oral reference dose (0.001 µg/g/day for Cd and 0.004 µg/g/day for Pb and 1.0 µg/g/day for Al\(^{15}\)), W is the average body weight (65 kg) and T is the average noncarcinogens’ exposure time (365 days/year × ED).

*Statistical analysis:* Due to the non-normal distribution of the samples, median values of each metal ± standard deviation were determined for each eggs’ type and compared with each other using Kruskal Wallis test to determine the significance (P<0.05). All the data were analyzed using SAS\(^{14}\).
Results and Discussion

The principle objective of this study was to assure the safety of hens’ eggs produced in an organic environment in comparison to other husbandry systems. This was accomplished through determining their containment of extra permissible levels of some major toxic elements. Since samples of this study were found to be non-normally distributed, medians of metals contents of examined eggs types were employed in this study (table 1). Different significant variations between types of eggs were noticed. CCE was significantly higher than OE and CFE (P< 0.05) in Cd levels, while there was no significant variation between the last two types.

Table (1): Statistical analysis of toxic metals (mg/kg) in examined egg samples and their respective THQ

<table>
<thead>
<tr>
<th>Metal (LOD, ppm)</th>
<th>Egg type</th>
<th>Samples &gt; LOD (n)</th>
<th>Range</th>
<th>Median</th>
<th>Quartile 3</th>
<th>THQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium (0.0006)</td>
<td>OE</td>
<td>43</td>
<td>0.01-0.108</td>
<td>0.046&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.065</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>CFE</td>
<td>50</td>
<td>0.02-0.108</td>
<td>0.067&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.086</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>CCE</td>
<td>50</td>
<td>0.11-0.276</td>
<td>0.222&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.241</td>
<td>0.22</td>
</tr>
<tr>
<td>Lead (0.02)</td>
<td>OE</td>
<td>25</td>
<td>0.04-0.376</td>
<td>0.251&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.304</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>CFE</td>
<td>35</td>
<td>0.04-0.452</td>
<td>0.343&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.411</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>CCE</td>
<td>50</td>
<td>0.03-0.458</td>
<td>0.284&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.324</td>
<td>0.07</td>
</tr>
<tr>
<td>Aluminum (0.01)</td>
<td>OE</td>
<td>50</td>
<td>1.76-6.76</td>
<td>4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.26</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>CFE</td>
<td>50</td>
<td>2.38-6.124</td>
<td>4.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.78</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>CCE</td>
<td>50</td>
<td>0.17-5.18</td>
<td>3.97&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.273</td>
<td>0.004</td>
</tr>
</tbody>
</table>

<sup>abc</sup>Medians with different letters are significantly varied (p<0.05)

On the contrary, significant variations were noticed between each eggs’ type for both Pb and Al residues. Whereas CFE was the highest in Pb residues (0.343 mg/kg), OE was the lowest (0.251 mg/kg). Interestingly, OE samples were found to have the highest level of Al (4.95 mg/kg). Unfortunately, available literature regarding toxic elements’ containment of hens’ eggs in relation to husbandry systems is quite scarce. However, Giannenas et al. had analyzed similar types of hens’ eggs produced at Greece and reported different significance pattern regarding Cd, where OE samples were found to have the highest amount. Another report comparing between different Latvian hens’ housing systems has revealed that OE samples had contained the highest level of Pb in contrast to our findings.

By interpretation of the obtained results, and in order to define pollution sources, CFE samples were found to have the highest median of Pb. This would be certainly attributed to excessive environmental rather than feed pollution. However, the maximum Pb and Cd concentrations in samples were belonged to CCE. Cd as a metal reach to birds feed mainly through environmental pollution which in turn reach to human food. Regarding Al, the highest median was belonged to OE samples. Al is found abundantly in soil rather than feed, and this may interpret the high level of Al in OE as hens of this type have the highest access to soil. Hens’ feed (water, additives, etc.) and husbandry system are the main influencing factors governing toxic element levels in eggs. In contrast to OE and CFE, CCE producing hens are not permitted to forage through soil or grass as they are fully caged in batteries. Accordingly, feed is considered the main factor in this husbandry system.
Correlation analysis of examined toxic metals among organic eggs samples yielded non-significant relationship between either of tested metals (figure 1). This certainly been attributed to the different sources of each metals

![Figure 1: Relationship between toxic metals in examined organic eggs samples using Spearman rank correlation. a) lead and cadmium; b) lead and aluminium and c) cadmium and aluminium](image)

Health hazards associated with consumption of toxic metals were described elsewhere\(^1,2,5\). Considering associated toxic hazards with consumption of examined eggs, two risk assessment analysis models were used; the maximum permissible limit and the THQ. Health and regulatory authorities defined certain levels for toxic elements, which should not be exceeded in different foods. Referring to Pb and Cd, regulatory authorities have set maximum levels of these elements in different foods\(^4\). Unfortunately, no maximum levels of both metals were assigned to eggs. Thus, in this study, we compared our findings with the maximum levels of nearby foodstuffs (poultry meat) in order to estimate the seriousness of these toxic elements in tested eggs.

Although OE was found to be the most complied type with the permissible limit standards of Pb and Cd (table 2), incidence of OE samples that have over permissible limits is alarming. 34 and 40% of OE samples were found to exceed permissible limits of Cd and Pd, respectively. This figure will extremely aggravate if compared with the maximum limit of Pb for raw milk (0.02 ppm), as 100% of all eggs samples would exceed this limit. While for Al, unfortunately, no clear level could be found to be the maximum allowed level in foods.

**Table (2): Compliance of examined eggs samples to maximum permissible limits of toxic metals in food.**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Permissible limits mg/kg in poultry meat*</th>
<th>Over permissible limits No. (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OE</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.05</td>
<td>17 (34)</td>
</tr>
<tr>
<td>Lead</td>
<td>0.1</td>
<td>20(40)</td>
</tr>
</tbody>
</table>

* EC, 4.

In respect to THQ, which is the most suitable parameter for assessment of health risks associated with toxic metals in food and soil\(^7\), results interpretation depends on the obtained THQ values. The closer the value toward zero, the lesser the risk of health hazards is probable. THQ value of more than 1 is pointing out potential risks. THQ calculation depends on various parameters which in turn greatly affect its credibility. Among them, the frequency of ingestion of food plays a significant role in figuring THQ value. As for our study, eggs consumption differed greatly among different countries depending on different criteria, like economical level, food and social habits and eggs production quantities. In Egypt, published per capita eggs consumption studies were not found. While for other countries, eggs consumption
has great variations (for example, Mexico, 355; China, 344; Spain, 239 eggs per year,\(^\text{10}\)). Thus, to have a predictive value, we used a recent dietary guideline of daily consumption of one egg per day\(^\text{13}\). Obtained THQ levels were all under 1, whereas we may conclude the lack of associated health risk (table 1). Even though, THQ levels of examined eggs did not solely rule these health risks as the sum of THQ of different food items should be taken into account. In another viewpoint, each type of eggs corresponds to a significant contribution of the total THQ (sum of THQ of each metal in a single type of eggs). Whilst CCE has a total THQ of 0.29, OE has the least of 0.12. Nevertheless, to better understand these values, we should take into our consideration the significant contribution of other sources and foods.

**Conclusion**

A thorough reconsideration should be paid for organic certifications of many foodstuffs like eggs, in which their production is affected by many conditions rather than feed. In addition, the concerned regulatory authorities should pay more attention to the chemical safety of all commercially available eggs types. As a specific recommendation, whereas organic eggs production regulations seemed to be not adequately appropriate, particularly in terms of toxic metals’ containment, eggs maximum levels of toxic elements should be distinctly defined.

**References**


