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Research Article

Comparative Study of Heavy Metal Concentration in Eggs Originating from Industrial Poultry Farms and Free-Range Hens in Kosovo

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The aim of the present study was to determine the most significant heavy metal concentration in hen eggs, in particular to compare the heavy metal concentration in eggs originating from industrial poultry farms versus free-range hens. The sampling process was carried out between October and December 2018, in the Republic of Kosovo. In total, 22 random egg samples were collected, with 54.5% and 45.5% of samples coming from poultry farms and free-range hens, respectively. The measurements of the heavy metals were taken by inductively coupled plasma mass spectrometry (ICP-MS), using a wide range of elements, and only the most important elements were reported, such as Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb within the range of 0.48–8.45, 38.77–289, 4286.59–15383.74, 0.45–144.74, 61.68–550.59, 1078.04–11378.56, 0.29–35.42, 0.27–6.54, and 0.04–1.41 $\mu g\,kg^{-1}$, respectively. This study revealed that the heavy metals in eggs from free-range hens are richer in essential elements such as Mn, Fe, and Zn, while poultry farm eggs contain a higher contribution of Cr, Cd, As, and Pb. However, the daily intake of heavy metals from egg consumption was lower than the WHO-FAO advised provisional daily intake. Therefore, egg consumption does not exceed the safety levels of toxic metals and does not pose any risk to human health.

1. Introduction

Hen eggs constitute high-value nutrients, cheap food for humans, and have an important role in the daily diet of humans globally. They are an important source of protein and contain several essential vitamins and minerals [1, 2]. Eggs are a good indicator of environmental contamination too [3, 4]. Mining, industry, domestic wastes, pesticides, and agricultural activities are responsible for significant high metal pollution in the environment [5, 6]. Heavy metals cannot be degraded or destroyed in the environment; they accumulate in the food chain through biotransformation and bioaccumulation [7]. Eggs are capable of accumulating heavy metals [8]. The frequent occurrence of heavy metals in eggs may be attributed to the contamination of hen eggs, which are exposed to heavy metals through feed intake and the surrounding environment [9]. The continuous intake of heavy metals through food at unsafe levels could have adverse effects in the terms of disrupting many biological and biochemical processes in humans, especially in children [10, 11].

Heavy metals have been categorized into essential and nonessential groups; metals such as chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), and zinc (Zn) are essential in small quantities for physiological and metabolic activities in living organisms [12]. The deficiency of any essential metal nutrients could result in many symptoms, such as the diseases of the immune system, various developmental abnormalities, and certain specific symptoms [13]. Other metals such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) are not essential, have no biological function, and may cause toxicity even in low concentrations. However, all heavy metals could be toxic if ingested in high concentrations [14, 15]. Chromium (Cr) is an essential element; if ingested in high concentrations, Cr is also mutagen teratogen and carcinogen [6]. Depending on the dose and the duration of exposure, as well as the age, gender, genetics, and nutritional status of exposed individuals, metals such as As, Cd, Hg, and Pb can be highly toxic metals and can affect the liver, kidneys, brain, and cardiovascular and reproductive systems [16]. A long-term intake of inorganic arsenic is closely associated with an increased risk of cancer in different parts of the human body, cardiovascular disease, and an increase in mortality [17, 18]. Cadmium is considered to be potentially carcinogenic and genotoxic and can accumulate in body tissues, particularly in the liver and kidney [19]. Heavy metals such as Pb and Hg are reported to have some neurotoxic effects which are especially harmful to the neurodevelopment of children [20]. Children are particularly susceptible to lead exposure due to high gastrointestinal absorption and the permeable blood-brain barrier [18]; long-term exposure to even small doses may cause anxiety, depression, restlessness, hypertension, anemia, damage to the foetal brain, tremors, kidney diseases, and autoimmunity diseases, such as rheumatoid arthritis [18, 21]. According to studies, a low-dose exposure to As, Cd, Pb, and Hg interactions in mixtures showed higher levels of toxicity than individual heavy metals [22].

The country of Kosovo is landlocked in the middle of southeastern Europe, covering an area of 10908 square kilometers (Figure 1), with a population of 1734 million [23]. According to the Statistical Yearbook "Kosovo Green Report 2017" of the Republic of Kosovo, this country with over 1.9 million hen eggs is an important source of egg production, and almost all of the egg products are consumed by people. Similar research was conducted in relation to honey [24].

In this study, we show the results of the determination of ten trace elements: chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb); specifically, we took into consideration elements recognized as toxic metals such as Cd, As, Pb, and Hg, including an essential element Cr, on eggs obtained from poultry farms and free-range hens.

Therefore, this study was carried out to estimate the quality of Kosovan eggs in terms of the concentrations of heavy metals, in order to assess their potential health risk to human beings, with regard to the daily consumption of hen eggs in the Kosovo region.

2. Materials and Methods

2.1. Sample Collection. This study covered the entire Kosovo region; the egg sampling was carried out between October and December 2018. A total of 22 random egg samples were obtained from different sources. Twelve samples came directly from breeding hens on poultry farms where mandatory food was supplemented by additions such as concentrates and balancers especially prepared by feed companies, and 10 samples came from free-range hens of noncommercial producers, where only natural food was used for feeding, such as cereals, legumes, scraps of food, nature supplies in the form of pasture, gravel, worms, bugs, and insects (Figure 1). One kilogram of eggs was collected from each farm; the yolks and whites of the egg samples were pooled and homogenized with an electric mixer in clean polyethylene bottles. The geographic coordinates for mapping were recorded by portable GPS. Subsequently, each sample was tagged and stored at −20°C until transported to the laboratory for chemical analysis. Prior to analysis, all egg samples were always weighed.

2.2. Chemical Analysis. The chemical analysis of elements was performed by ICP-MS, in high sensitivity mode, type Bruker 820 (Karlsruhe, Germany). ICP-MS was used successfully for multielements determinations in a single measurement. The conditions for the ICP-MS system were plasma flow: 18.00 l/min, sheath gas flow: 0.20 l/min, auxiliary flow: 1.80 l/min, nebulizer flow: 1.01 l/min, sampling depth: 6.50 mm, power: 1.40 kW, pump rate: 4 rpm, and stabilization delay 10 s [25].

2.3. Statistical Analysis. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences) software, version 21. The independent *t*-test was used for comparison purposes followed by the least significant difference (LSD) test. The level of significance for the differences was set at $p < 0.005$ and $p < 0.001$.

2.4. Estimated Daily Intake. The daily intake of metals is a fundamental parameter for health risk assessments [26]. The estimated daily intake depends on both the metal concentration in eggs and its daily consumption. It was found that the average per capita consumption of hen eggs in 2017 in Kosovo was 8.9 kg, equivalent to 24 g/d [27]. Based on this figure, the estimated daily intake (EDI) values were calculated by the following equation:

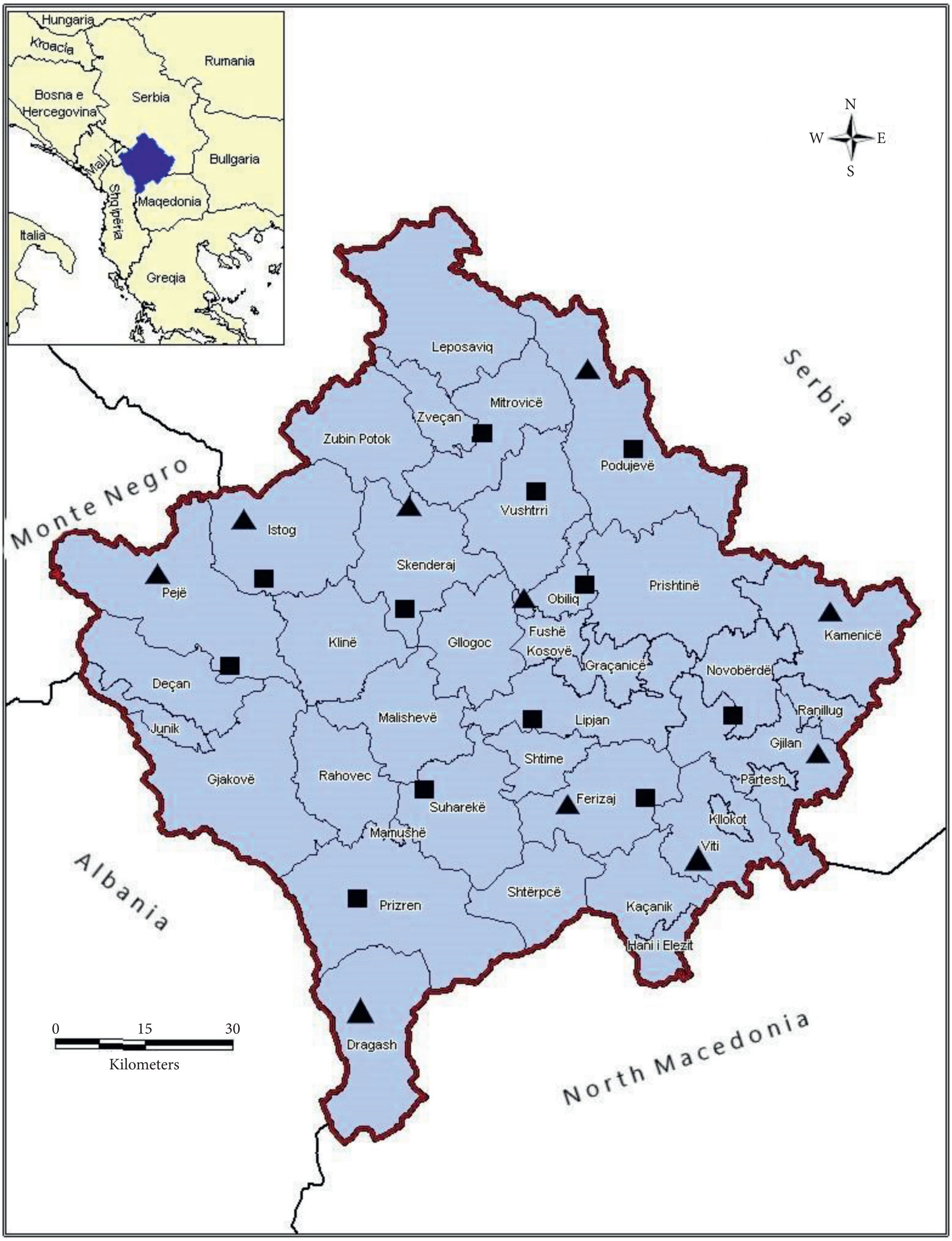
$$\mathrm{DI}_{\mathrm{HM}} = \mathrm{C} * \mathrm{A}, \quad (1)$$

where $\mathrm{DI}_{\mathrm{HM}}$ stands for the daily intake of heavy metals, C represents the concentration of metal in the egg (μg kg^{-1}), and A is the average per capita consumption of eggs (g/day).

3. Results and Discussion

The descriptive analysis of metal concentrations for Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb is presented in Table 1. In total, trace elements of Mn, Fe, Cu, Zn, and As were found in 100% of the examined egg samples, followed by Hg (77%), Ni (72%), and Pb (36%), while Cr and Cd presented with the lowest rate of 32% among the examined egg samples.

An average concentration of essential elements in whole eggs was found in the case of Mn ($154.49 \pm 74.26\,\mu$g kg^{-1}), Fe ($8220.55 \pm 2708.03\,\mu$g kg^{-1}), Ni ($18.28 \pm 34.72\,\mu$g kg^{-1}), Cu ($243.39 \pm 150.25\,\mu$g kg^{-1}), and Zn ($4222.32 \pm 2697.22\,\mu$g kg^{-1}). The study showed significant differences ($p < 0.001$) between the content of Mn, Fe, and Zn in hen egg samples which originated from poultry farms and free-range hens, whereas



Egg samples:
■ Poultry farm
▲ Free-range hens

Figure 1: Map showing the location of sampling egg site in Kosovo.

Table 1: Average concentrations and standard deviation of metals in hen eggs from poultry farms and free-range hens in parts per billion (μg kg^{-1}).

Elements/Eggs	Range (μg kg^{-1})	Mean ± SD (μg kg^{-1})	Element presented on samples (%)
Chromium (Cr)	0.48–8.45	3.58 ± 3.041	32
Poultry farms	0.85–8.45	5.03 ± 3.23	30
Free-range hens	0.48–3.33	1.65 ± 1.49	30
Manganese (Mn)	38.77–289.00	154.49 ± 74.26	100
Poultry farms	38.77–268.78	145.27 ± 73.00	100
Free-range hens	79.50–289.00	176.04 ± 69.00	100
Iron (Fe)	4286.59–15383.74	8220.55 ± 2708.03	100
Poultry farms	4286.59–11942.41	7894.50 ± 2105.63	100
Free-range hens	4749.38–15383.80	9179.85 ± 3411.90	100
Nickel (Ni)	0.45–144.74	18.28 ± 34.72	73
Poultry farms	2.83–144.74	25.63 ± 48.24	66
Free-range hens	0.45–144.80	26.65 ± 48.97	80
Copper (Cu)	61.68–550.59	243.39 ± 150.25	100
Poultry farms	61.68–517.52	251.30 ± 151.50	100
Free-range hens	64.08–550.59	265.92 ± 278.95	100
Zinc (Zn)	1078.04–11378.56	4222.32 ± 2697.22	100
Poultry farms	1078.04–7587.63	4043.50 ± 2622.76	100
Free-range hens	1452.31–11378.56	4923.24 ± 2952.40	100
Arsenic (As)	0.29–35.42	2.96 ± 7.34	100
Poultry farms	0.29–1.31	0.805 ± 0.33	100
Free-range hens	0.36–35.42	5.196 ± 10.70	100
Cadmium (Cd)	0.27–6.54	2.18 ± 2.57	32
Poultry farms	0.83–6.54	4.16 ± 2.97	25
Free-range hens	0.27–1.71	0.70 ± 0.68	40
Mercury (Hg)	0.04–1.41	0.34 ± 0.33	77
Poultry farms	0.07–1.41	0.39 ± 0.47	66
Free-range hens	0.04–0.52	0.28 ± 0.14	100
Lead (Pb)	12.85–71.80	32.92 ± 23.18	36
Poultry farms	35.51–71.80	57.40 ± 19.26	25
Free-range hens	12.84–26.43	18.22 ± 5.96	50

SD, standard deviation.

no difference was found between the content of Ni and Cu ($p > 0.005$). The present results in relation to Fe were similar to other studies carried out in Spain (7620 μg kg^{-1}) [28], whereas significantly higher concentrations were shown for Mn (360.0 μg kg^{-1}), Ni (96.00 μg kg^{-1}), Cu (640.0 μg kg^{-1}), and Zn (11640 μg kg^{-1}) compared to research studies carried out in Italy, Greece, and China [3, 29, 30].

Among the metals included in the study, significantly greater in the concentration of metals between the samples from poultry farms and free-range hens were observed for Cr, Cd, As, and Pb.

The average concentration of Cr in whole eggs was 3.58 ± 3.041 μg kg^{-1}. The mean concentration of Cr in eggs from poultry farms was significantly higher than the mean concentration in eggs from free-range hens ($p < 0.001$). The highest concentration of Cr (8.44 μg kg^{-1}) was found in eggs from poultry farms in the region of Ferizaj followed by Lipjan (6.40 μg kg^{-1}) and Vushtrri regions (4.44 μg kg^{-1}). The significantly higher concentration of Cr from poultry farms could be related to the fact that Cr is an essential element, and dietary Cr supplementation has been shown to have positive effects on growth performance, egg production, feed efficiency, and egg quality in poultry [6, 31, 32]. Our study results indicate that a similar tendency of Cr was reported by studies in Italy (8.0 μg kg^{-1}) [3], while higher levels were determined in studies conducted in Spain (70.0 μg kg^{-1}) and Taiwan (300.0 μg kg^{-1}) [28, 33].

The mean concentration of Cd in all egg samples was found to be 2.18 ± 2.57 μg kg^{-1}. The differences in Cd concentration between the poultry farms and free-range hens were found to be statistically significant ($p < 0.001$); the same pattern was also registered by Saad Eldin and Raslan [34]. The highest level of Cd was found in poultry farms in Vushtrri 6.54 μg kg^{-1} and Lipjan regions 5.12 μg kg^{-1}. The main exposure to Cd in the general population comes from food [18]. The Cd content in this study was lower than the amount found in studies in Egypt (90.0 μg kg^{-1}) and Romania (60.0 μg kg^{-1}) [34, 35]. The lowest mean concentration of Cd was reported in eggs from certain European countries, France (0.4 μg kg^{-1}), Greece (1.0 μg kg^{-1}), and Italy (3.0 μg kg^{-1}) [3, 30, 36].

The average concentration of As in all egg samples was found to be 2.96 ± 7.34 μg kg^{-1}. As concentration was higher in the eggs of free-range hens than in those from poultry farms, as shown in Table 1. Industrial development, such as mining, is considered to be the major source of air pollution resulting from As [37]. The highest concentration of As (35.42 μg kg^{-1}) was detected in one egg sample from a domestic farm in the region of Kamenica. The presence of a higher concentration of As was expected for this region

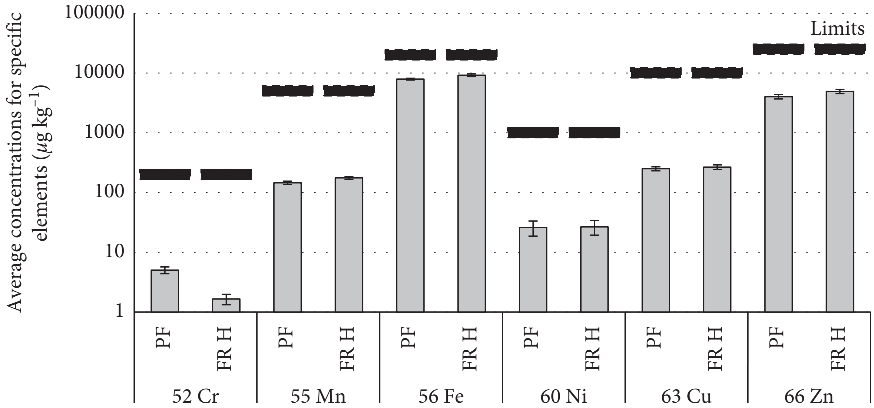


FIGURE 2: Estimated daily intake (μg kg^{-1}/g/day) of Cr, Mn, Fe, Ni, Cu, and Zn from hen eggs according to the FAO/WHO provisional tolerated daily intake. PF, poultry farms; FR H, free-range hens.

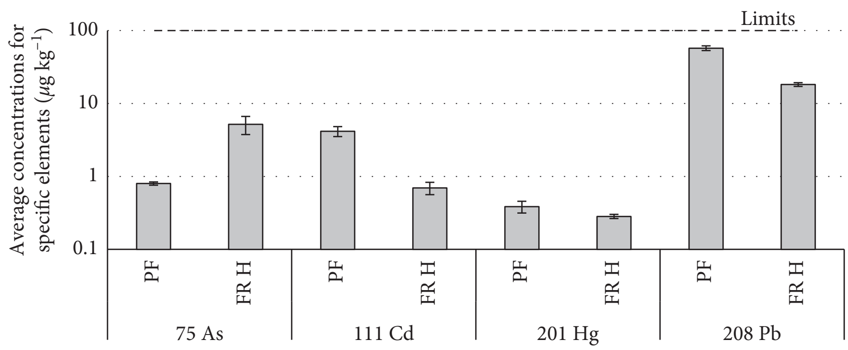


FIGURE 3: The calculation of average daily intake (μg kg^{-1}/g/day) of As, Cd, Hg, and Pb from hen eggs according to maximum permissible limits (MPL) established from the FAO/WHO. PF, poultry farms; FR H, free-range hens.

compared to other regions, due to the fact that this region is located near the mine complex in the city of Kamenica. Reported studies showed that As values are relatively low, especially when compared with the data from Italy (7.0 μg kg^{-1}) and Alaska (126.0 μg kg^{-1}) [3, 8].

Regarding the Pb element, the average concentration in whole egg samples was $32.92 \pm 23.18\,\mu$g kg^{-1}. The mean concentration of Pb in eggs from poultry farms was significantly greater than the averages of free-range hens ($p < 0.001$). The same pattern in average Pb levels was recorded in Romania [35]. The highest concentration of Pb was found in eggs from poultry farms in the region of Vushtrri (71.80 μg kg^{-1}), Lipjan (64.87 μg kg^{-1}), and Obiliq (35.52 μg kg^{-1}), respectively. Fakayode and Olu-Owolabi found significant positive correlations between the levels of metals in feed and the corresponding levels of metals in the eggs [1]. The highest rate in free-range hens was found in the Obiliq (26.44 μg kg^{-1}) and Skenderaj regions (22.24 μg kg^{-1}). The high Pb concentration in samples from free-range hens can be explained due to the presence of the large metallurgic and mining complex (Trepca) in this region, which is also considered to be the main source of environmental pollution. The overall average concentration of Pb in this study was different from the averages reported in eggs from Italy (19.0 μg kg^{-1}), China (52.0 μg kg^{-1}), India (489.0 μg kg^{-1}), and Nigeria (590.0 μg kg^{-1}) [1, 3, 38, 39].

Mercury is a hazardous environmental contaminant and is very toxic in relation to the human embryo and foetus [6, 20]. The mean concentration of Hg in all sample eggs was found to be $0.34 \pm 0.33\,\mu$g kg^{-1}. The levels of Hg detected in egg samples from the poultry farms were slightly higher than that found in free-range hens. The content of Hg was much lower than the concentrations reported to be found in eggs from China (0.60 μg kg^{-1}) and Canada (4.00 μg kg^{-1}) [6, 38].

3.1. Estimated Daily Intake of Heavy Metals through Eggs. The daily intake of essential elements such as Cr, Mn, Fe, Ni, Cu, and Zn and nonessential elements such as As, Cd, Hg, and Pb were calculated and compared with the advised provisional and recommended daily intake (RDI) of minerals [40] and maximum level (ML) established by the FAO/WHO for certain foods [41].

The calculated data of the average daily intake of Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb, based on hen egg consumption, did not exceed the standard limit, as shown in Figures 2 and 3. From the essential elements group, Fe and Zn reached closer to the limit compared to other group elements. The eggs originated from free-range hens contain higher Fe and Zn comparing to the eggs originated from poultry farms as shown in Table 1. While, from the toxic heavy metal concentration group, shown in Figure 3, Pb reached the highest level, which is not too far away from the standard limit.

Widely, the toxicity of Pb is known. The adults take around 10–15% of lead from the food, while children may absorb up to 50% of lead via gastrointestinal tract; also, the rate of inorganic lead successfully can cross blood-brain barrier; this coefficient is much higher to the children, and this made them more sensitive than adults [18]. The eggs originated from poultry farms contain higher concentrations of Pb (57.4 ± 19.26) comparing to the eggs originated from free-range hens (18.22 ± 5.96). Probably, the food and water of hens are affecting this big difference of Pb concentrations, and further studies should be taken to prove this difference.

4. Conclusion

The present study has shown the differences between the trace element content of hen egg samples originating from poultry farms and from free-range hens. The eggs from free-range hens showed that they are richer in essential elements Mn, Fe, Ni, Cu, and Zn, while poultry farm eggs contained a higher contribution of Cr. However, the presence of toxic metals, namely, Cd, As, and Pb, was higher in poultry farm eggs than free-range eggs. This may be due to contamination of the food composition used as hen feed, as a result of pesticides and the activities of industrial factories. Finally, the elevated levels of heavy metals especially Pb need further monitoring of food composition used as hen feed, particularly in industrial regions.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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