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Determination of heavy metals from table poultry eggs in Peshawar-Pakistan

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Abstract
Environmental problems related to pollution of soil, food and water have been increased with industrialization. This work aims to quantify the heavy metals (Cd, Cu, Cr, Pb, and Zn) concentration of edible poultry eggs which directly affect human health. Twelve consecutive eggs from same bird in triplicate were collected from five different poultry farms i. e. F_1, F_2, F_3, F_4 and F_5 in district Peshawar Khyber Pakhtunkhwa. Total concentration of egg parts egg white, egg yolk and whole egg basis was determined by atomic absorption spectrophotometer. Results indicated that on whole egg basis the Cd, Zn and Pb content showed 2-3fold increases than the permissible daily intake level. However the Cu was within consumable limit while Cr was undetectable. It results obtained can be used as primary tools to further ensure egg quality control and also can work as dataset for the close monitoring of food contamination by governments and environmentalist.

Keywords: poultry, egg, heavy metal, carcinogens

1. Introduction
The birds are bred to fulfill the ever increasing requirement of animal protein for the majority of the world population. During the last two decade, many developing countries have adopted intensive poultry production to meet the demand of animal protein. The intensive poultry farming is seen as a way to quickly increase the supply, economical, palatable and healthy food protein for growing urban populations [1]. The poultry industry is now a largest sector of agriculture throughout the world and is rapidly increasing as industry in South Asia. In Pakistan, poultry farming has increasing share in the national economy and is currently providing about 1.5 million people employment. Export of live poultry and meat from Pakistan increased from Rs.27 million in 2009-10 to Rs.1.08 billion in 2010-11. In the financial year 2012-13, government fixed 13,813 million tonnes egg production target however, the egg production already has surpassed this target by 6.5% increase [2].
Fresh eggs are among the most important and nutritious food in the daily diet. Moreover, eggs are included in several food products for different functions [3, 4]. Global environment is polluted with heavy metal which leads to an increased interest in metal contamination of food stuffs and amongst them eggs which represent an important part of human's diet especially in children [5]. Despite considerable interest in the trace element contents of eggs by poultry breeders, nutritionists and environmental scientists, available data about trace elements levels in eggs are scarce [6].
At present poultry farming is being concentrated around the large urban centers all over the country. All mineral elements present in poultry feed at higher amounts can pose several adverse health issues to poultry products. Currently, industries wastes as well as other wastes are being added to environment untreated. Therefore, it is essential that heavy metals must be restricted in the diet at the adequate levels in poultry products to ensure consumer health. A few research has been carried out at National research council (NRC) for both, dietary maximum tolerable levels of these elements in poultry feed as well as maximum tolerable levels (MTL) concentrations of these metals in dietary poultry eggs [7].
Metallic elements are found in all living organisms where they play an important role, as structural, components of control mechanisms (e.g. in nerves and muscles) and enzyme activator. Some metals are essential as copper (Cu), zinc (Zn), calcium (Ca), if present in permissible limits play a perfect role in the intrinsic mechanisms regulating vital biological processes [8].

Whereas non essential metals especially lead (Pb) and cadmium (Cd) are toxic even in trace amount [9]. Essential elements (zinc and copper) deficiency results in mutilation of biological function, but when their intakes exceed the recommended quantities eye and skin lesions and sexual maturation are more severe cases [10, 11]. Quantification of the heavy metals, such as Cd, Pb, Cu and Zn in the poultry eggs is very important due to their essential function as well as toxicity to the consumer. Therefore, monitoring of these metals in table eggs is important due to their carcinogenic effect on consumer which can pose serious threats to liver and kidney [12].

Therefore, present study was conducted for simultaneous determination of the Zn, Cd, Pb and Cu in poultry table eggs to quantify the risk contamination from five representative poultry farms in and around Peshawar-Pakistan.

2. Materials and methods

2.1. Reagents and solution preparation

All reagents used in the study were of analytical grade from (Merck, Darmstadt, Germany). Stock solutions of 1000 mg/l of each Zn, Cd, Pb and Cu were prepared by dissolving appropriate amount of cadmium nitrate, copper nitrate, lead nitrate and zinc chloride in water and diluted to 1000 ml volumetric flask. Ultrapure deionized water was used throughout the study.

2.2. Egg sampling and sample preparation

In November, 2012, fresh egg samples were collected from five layers farms (12 eggs from each farm) in Peshawar were collected and transferred in plastic bags to the laboratory and stored at dark cool place. Each egg was cut in the air cell end using pointed forceps and dissecting scissors (cleaned with soap and rinsed with distilled water) for each egg separately. The content of each sample was placed in a chemically cleaned glass jar and egg white was separated from egg yellow. Samples egg white and yolk was dried at 75°C to get constant weight [13].

2.3. Sample digestion and analysis

One gram of samples was digested with 20 ml of nitric acid (HNO_3) in 250 ml Erlenmeyer flask and placed over night and next day boiled on hot plate for 5 min. Add 10 ml of sulphuric acid (H_2SO_4) and the digestion process was continued until the solution become clear. After this samples were transfer into volumetric flask and filled up to 100 ml with distilled water. Heavy metals (Zn, Pb, Cu, Cd and Cr) were determined with the help Atomic absorption spectrophotometer (model Perkins Elemer 2000) by using standard method [14].

2.4 Statistical analysis

The data regarding the extent of heavy metals in each egg part were analyzed using analysis of variance followed by statistical software package Statistica 12 and figures were made.

3. Results

The results obtained for analysis of Cd, Cu, Pb and Zn from all five poultry forms are presented in figure 1, where as the comparison was made with maximum permissible limits known worldwide and values are given in table 1 [15, 16]. The results were compared in egg white, egg yolk and on whole egg basis.

3.1 Cadmium

Results regarding cadmium content in all egg parts (egg white, egg yolk and whole egg) obtained from different farms have been given in figure 1a. There was significant ($p < 0.05$) difference in Cd content in all three egg parts. The Cd concentration in eggs among all farms ranged from 0.021–0.024 mg kg^{-1} in egg white, 0.021–0.063 mg kg^{-1} in egg yolk and 0.042–0.087 mg kg^{-1} on whole egg basis, respectively (Fig. 1a). On whole egg basis, the Cd concentration was much higher in than the permissible international limit of 0.05 mg kg^{-1}.

3.2 Copper

The all egg parts (egg white, egg yolk and whole egg) showed increased level of Cu concentration (Fig. 1b). Comparison was made among all farms and the relative increase in Cu content in egg yolk was 15.32% to 111.32% on whole egg basis when compared to the permissible limit (Table 1).

3.3 Lead

The data showing the Cu contents among all farms eggs studied is summarized in Figure 1c. The Cu content ranged from 0.025–0.119 mg kg^{-1} in egg white, 0.086–0.345 mg kg^{-1} in egg yolk and 0.058–0.464 mg kg^{-1} on whole egg basis. However, the Cu contents did not show increased level than the permissible limits of 0.5 mg kg^{-1}.

3.4 Zinc

Similar to the Cu, the Zn content did not showed increased level than the permissible limit as described in table 1. The Zn content in different egg parts ranged from 0.022–0.052 mg kg^{-1} in egg white, 0.09–0.14 mg kg^{-1} in egg yolk and 0.046–0.166 mg kg^{-1} on whole egg basis (Fig 1d). The chromium content was undetectable in the egg samples.

Table 1. Permissible limits of heavy metals in table hen eggs.

Metal	Permissible Limit (mg kg^{-1})
Cadmium	0.05[a]
Copper	10[a]
Chromium	0.002[b]
Lead	0.5[a]
Zinc	20[a]

Source: ([a]Zmudzki and Szkoda 1996; [b]Roychowdhury *et al.* 2003)

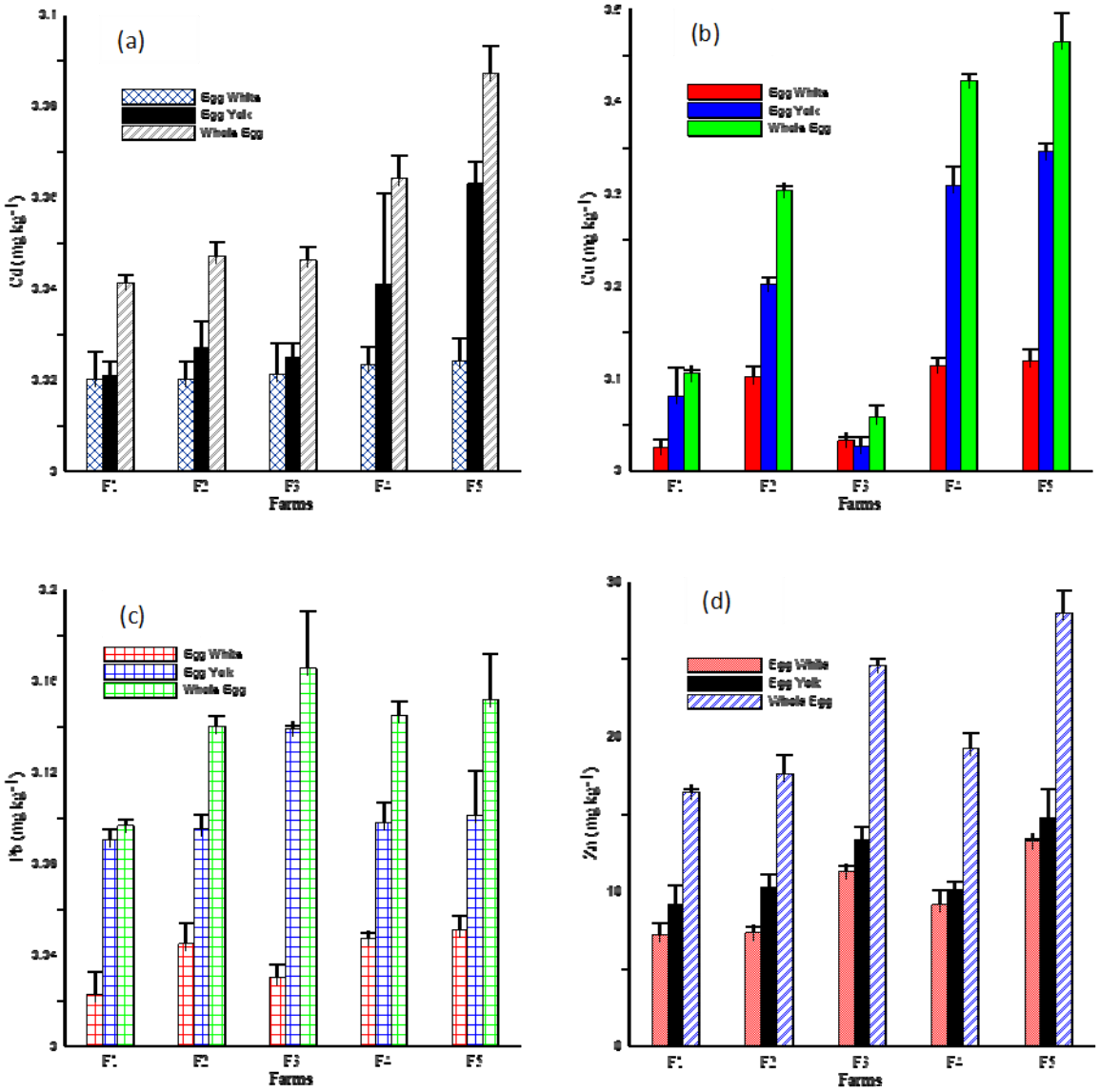

Fig 1: (a) Total cadmium (Cd) content in egg parts; (b) total copper (Cu) content in egg parts; (c) total lead (Pb) contents in egg parts and (d) total zinc (Zn) content in egg parts. The horizontal bars show values as mean ± SD (n=12).

4. Discussion

Determination of heavy elements in table poultry eggs can become an important tool for food nutritionists and environmentalists. Our study aimed to provide a baseline of the composition of domestic eggs with special reference to heavy metal concentration as such information had been previously lacking in the Peshawar region. The increase concentration of Cd and Pb was higher than the permissible limits in the all sample analyzed. Many researchers have reported that the increased content of the heavy metals is contributed the high intake of these elements by birds from feed and water. According to [17, 25] increase in cadmium content of egg has a positive correlation with increased cadmium content of feed. In another study from [15] also showed the similar trend. The recent review by [17] gives a general insight on transfer of toxic substances from feed to eggs. According to [18] Cd concentrations were below the limit of quantification for the majority of egg samples. However, [19] recorded that average concentration of Cd in eggs was 0.07 mg kg^{-1} which was comparatively greater than levels found in other local and international standards cited by [15].

The results releated to Cu, Pb, and Zn levels in chicken egg are in accordance with those reported by Ferguson et al. [20]. Our measurements for Cu and Pb content in eggs were lower than those recorded by Hui [21]. However, the eggs obtained from an area over exploited by pollution and unfortunately, no further data from other poultry species were available to clarify this discrepancy.

Environmental concerns have increased the need for determination of trace elements in eggs of domestic and free-living birds [22, 23]. In order to explain pollution effects on poultry products, many studies have been conducted on the embryotoxic effects resulting from metal pollution. It is known that the chicken can control over metal depositions into the egg by avoiding mineral deposition [24, 25]. However, it has been reported that, although these multiple layers of protection maybe adequate for some minerals such as Cr and Mn, they may be inadequate for others such as Pb in the egg [21].

An improved understanding of the content of egg trace element levels that are naturally achieved by commercial

poultry kept under unpolluted sites can provide reference points for pollution effects and also guidance in formulating dietary supplements for commercialized poultry birds. This need for a reference population from unpolluted sites is emphasized by the natural interspecies variability in yolk and albumen Cd, Pb and Cr levels reported in some innovative investigations [15, 25].

5. Conclusions

It is concluded that the table poultry eggs contaminated from heavy metals, Cd and Pb was bio-accumulated in egg yolk and albumen. The toxic elements Cd and Pb are of particular concern as most of the detected concentrations in the eggs exceeded both local and international guideline values. Their effect on the local population due to prolonged consumption of these contaminated eggs is unknown and deserves further detailed investigation of water and feed used to feed these poultry birds.

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