

QUANTIFICATION OF HEAVY METALS IN LAYER AND DOMESTIC HEN EGGS

Original Research

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ABSTRACT

Background: Poultry products are a crucial source of nutrition, especially in developing countries where they significantly contribute to addressing protein deficiencies. However, the prevalence of heavy metal contamination in these products poses a public health concern, with heavy metals entering the poultry supply chain through environmental pollution, contaminated feed, and inadequate handling practices. This study focuses on Pakistan's rapidly growing poultry industry and the risks posed by heavy metals such as lead, cadmium, arsenic, and mercury in poultry products.

Objective: To evaluate the levels and sources of heavy metal contamination in eggs and poultry products in Pakistan, assess associated health risks, and recommend regulatory measures for minimizing exposure.

Methods: Egg, water, and feed samples were collected from local markets, poultry farms, and student research projects within the Multan district. Samples were stored at room temperature in sealed containers before analysis. Wet acid digestion was used to prepare samples, followed by atomic absorption spectroscopy (AAS) to quantify heavy metal concentrations. Levels of lead (Pb), cadmium (Cd), arsenic (As), zinc (Zn), cobalt (Co), iron (Fe), and copper (Cu) were measured and compared across domestic and layer hen eggs.

Results: Significant differences were found in heavy metal levels between domestic and layer hen eggs. Domestic eggs contained higher Pb levels ($0.830 \pm 0.011 \mu\text{g/g}$) compared to layer eggs ($0.043 \pm 0.014 \mu\text{g/g}$). Cobalt levels in domestic egg whites reached $2.369 \pm 0.302 \mu\text{g/g}$, surpassing the $1.138 \pm 0.023 \mu\text{g/g}$ detected in layer eggs. Copper concentrations in domestic eggs were notably higher, at $0.360 \pm 0.027 \mu\text{g/g}$ in the yolk, compared to layer eggs. However, cadmium and arsenic levels remained low across both groups.

Conclusion: Heavy metal contamination in poultry products poses significant health risks, especially for vulnerable populations. Regulatory measures, improved feed and water quality, and stringent environmental monitoring are essential for minimizing exposure and supporting public health while enabling sustainable growth in Pakistan's poultry industry.

Keywords: Arsenic; Cadmium; Eggs; Food Contamination; Food Safety; Lead; Poultry.

INTRODUCTION

Poultry and its products, particularly eggs, are recognized as crucial sources of essential nutrients, offering a well-balanced supply of proteins, vitamins, and minerals, which are especially valuable in child nutrition. Poultry farming has emerged as a fundamental component in meeting the nutritional needs of Pakistan's population, reflecting a substantial annual growth rate between 15% and 20%, with approximately 200 billion invested in this sector. In 2011-12, the poultry population included around 721 million birds, generating over 13 billion eggs and a significant portion of the national poultry meat supply. This growth has contributed to Pakistan's economy, with egg and poultry exports rising from Rs.27 million in 2009-10 to Rs.1.08 billion in 2010-11, providing direct employment for about 1.5 million individuals (2, 5, 6). The growing demand for protein-rich foods like eggs, along with an expanding agricultural sector, highlights the sector's potential to address nutritional needs while promoting economic growth.

Despite these benefits, food safety remains a pressing global issue, with over 420,000 deaths yearly due to foodborne illnesses, nearly a third of which involve children under five. According to the World Health Organization (WHO), contaminated food consumption results in an estimated loss of 33 million disability-adjusted life years (DALYs), and the true burden is likely even higher. This urgency underscores the need to address food safety threats, such as heavy metal contamination in poultry products like eggs, to prevent adverse health effects (7). Heavy metals—elements with high atomic weights and densities—pose significant health risks even at low concentrations due to their non-biodegradable and thermodegradable nature. They persist in the environment and can bioaccumulate within the food chain, eventually reaching consumers. Critical metals, including iron (Fe), zinc (Zn), nickel (Ni), copper (Cu), cadmium (Cd), mercury (Hg), arsenic (As), and lead (Pb), become particularly harmful when present beyond safe levels in food. Their presence has been associated with numerous adverse health outcomes, including cancers, organ damage, metabolic disorders, and skeletal and hormonal disturbances (8, 10, 11).

In developing countries like Pakistan, heavy metal contamination in poultry products may stem from environmental pollution, primarily due to industrial discharge and untreated sewage used in irrigation. Exposure pathways for these metals include ingestion, inhalation, and dermal contact, affecting both urban and rural areas. Rural poultry farmers often allow poultry to roam near contaminated water sources, which increases the risk of metal accumulation in the birds. Mismanagement of animal feed, farm equipment, and contaminated containers further exacerbates the issue, as does the lack of awareness and inadequate regulatory enforcement in developing regions (13, 14, 15, 16).

International studies have consistently shown that developing countries exceed acceptable levels of heavy metals in poultry products, primarily due to insufficient food safety regulations and poor handling practices. Lead (Pb) is one of the most prevalent heavy metals detected in eggs, with levels in certain countries surpassing the Codex Alimentarius Commission's permissible limit of 0.02 mg/mL. Pb exposure, which can occur through ingestion or inhalation, is linked to neurotoxicity, renal failure, reproductive issues, and other systemic conditions. Eggs and poultry from Egypt, Serbia, and Poland have shown Pb contamination well above acceptable limits, while developed nations generally report lower contamination due to stricter regulations and more advanced detection methods (18, 19, 20, 21, 22).

Cadmium (Cd), another hazardous metal, primarily targets the kidneys and liver, leading to potentially fatal health effects. Sources of Cd contamination include phosphate fertilizers, fossil fuel combustion, and certain industrial practices. In countries like Pakistan, Nigeria, and Egypt, Cd levels in eggs frequently exceed international safety standards, underscoring the need for stringent monitoring and control measures. In contrast, developed nations like Spain, Croatia, and Korea report minimal Cd levels, attributed to effective regulatory practices (25, 26).

Arsenic (As) contamination, similarly, poses severe health risks, including cancers, digestive issues, and neurological disorders. In Pakistan, arsenic levels in poultry products have exceeded safe thresholds, suggesting a pressing need for regulations to limit As in poultry feed and water sources. Mercury (Hg) contamination, particularly its methylmercury form, further threatens food safety, as it bioaccumulates through the food chain, leading to nervous system damage and other health issues in humans. The Minamata Convention on Mercury has highlighted the global effort required to reduce Hg emissions, focusing on high-risk food sources like poultry (27, 28, 32, 33, 34, 35, 36).

Given these findings, the objective of this study is to examine heavy metal levels in poultry products, such as eggs, with a focus on identifying contamination sources and assessing associated health risks. By investigating the prevalence of heavy metals in eggs and proposing mitigation strategies, this study aims to support safe consumption choices and contribute to food safety standards that protect public health while fostering the economic growth of the poultry industry in Pakistan (12, 37, 38).

METHODS

Egg samples were collected from various locations across the Multan district, including local markets, shops, and homes, while feed samples were obtained from students conducting research on heavy metals within the same department. Each sample was carefully placed in zipper-sealed bags and stored at room temperature in the Food Safety and Microbiology Laboratory at Bahauddin Zakariya University (FFSN, BZU) to maintain sample integrity before analysis. To assess heavy metal intake through water consumption, water samples were collected from poultry farms where birds are raised, and these samples were preserved in transparent glass bottles at room temperature (26).

Heavy metal analysis of aqueous samples, including water and egg samples, required an appropriate level of detection and analyte-specific methodologies. Due to the high organic matter content in egg samples, a predigestion step was essential to prepare the samples effectively. Acid digestion, also known as wet digestion, was selected as the preferred predigestion technique, as it is generally more efficient than alternatives such as dry digestion or ashing (1).

The wet digestion process began by measuring one milliliter (mL) of each sample with a micropipette, ensuring precision and accuracy. The weight of the aluminum foil covering each flask was also measured to eliminate potential errors. Following sample measurement, ten milliliters (mL) of concentrated nitric acid (HNO_3) was added to each flask, which was subsequently covered with aluminum foil and stored in a dark environment for 24 hours. After this initial period, five milliliters (mL) of perchloric acid (HClO_4) were introduced to each flask, and the samples were left undisturbed for an additional four hours.

To complete the digestion, the samples were then placed on a hot plate set to 200°C until they exhibited a translucent appearance, signifying the effective breakdown of organic matter within the sample matrix. After digestion, each sample was diluted to a final volume of 50 mL using double-distilled water to ensure consistency across samples. The cooled samples were then filtered using Whatman filter paper No. 42 to remove any residual particulates, enhancing the purity of the final preparation.

Following filtration, the prepared samples were stored in sealed bottles within the laboratory, awaiting further analysis. Heavy metals were subsequently quantified using atomic absorption spectroscopy (AAS), with the flame atomic absorption spectrophotometer providing a reliable, cost-effective, and user-friendly method for detecting heavy metal levels in egg samples. This method is widely favored in developing nations for its efficiency and ease of operation (25, 17).

RESULTS AND DISCUSSION

The analysis of heavy metal concentrations in layer and domestic hen eggs revealed notable variations in levels of lead (Pb), cobalt (Co), and copper (Cu) between these two categories of eggs, indicating potential environmental contamination differences. Atomic absorption spectroscopy (AAS) was employed for heavy metal detection, with flame atomic absorption spectrophotometry (FAAS) serving as the preferred method due to its cost-effectiveness, rapid detection capability, and ease of handling (37). Lead was found in significantly higher concentrations in domestic hen eggs than in layer eggs, suggesting a greater susceptibility of domestic hens to environmental lead exposure. Specifically, the Pb concentration in the egg whites of domestic hens was recorded at $0.830 \pm 0.011 \mu\text{g/g}$, a stark contrast to the much lower concentration of $0.043 \pm 0.014 \mu\text{g/g}$ observed in layer eggs. This marked difference emphasizes the need for further examination of environmental factors contributing to elevated lead levels in domestic hen eggs.

	Egg Part	Pb	Cd*	Zn	Co	As	Fe*	Cu
Layer	White	0.043±0.014c	0.030±0.015	0.763±0.097c	1.138±0.023d	0.061±0.010a	0.492±0.054	0.149±0.011d
	Yolk	0.024±0.006c	0.021±0.010	0.863±0.076b	1.901±0.081b	0.013±0.004c	0.636±0.036	0.176±0.013c
Domestic	White	0.830±0.011a	0.033±0.012	0.968±0.085a	2.369±0.302a	0.033±0.010b	0.790±0.042	0.302±0.048b
	Yolk	0.668±0.214b	0.027±0.014	0.738±0.101c	1.651±0.107c	0.013±0.004c	0.919±0.044	0.360±0.027a

Cobalt (Co) and copper (Cu) concentrations also displayed substantial variation, with domestic hen eggs showing higher Co levels in the white portion (2.369±0.302 µg/g) compared to layer eggs (1.138±0.023 µg/g). Copper levels in domestic hen yolks reached 0.360±0.027 µg/g, indicating variations between the two egg types. The presence of these metals, particularly Co and Cu, implies that environmental pollution surrounding domestic poultry environments may have a more pronounced impact on the heavy metal content of these eggs. Interestingly, the concentrations of cadmium (Cd) and arsenic (As) were minimal and similar across both types of eggs, suggesting that these metals did not pose significant contamination concerns in this context. However, zinc (Zn) levels varied, with a higher concentration in the albumen of domestic eggs, while yolk served as the primary Zn reservoir in layer eggs. This distribution may reflect differences in feeding practices or environmental exposure in different rearing settings.

Iron (Fe) levels appeared relatively consistent between layer and domestic hen eggs, indicating that environmental factors influencing Fe bioaccumulation may not differ substantially between these poultry types. The observed disparities in metal concentrations, particularly Pb, Co, and Cu, underline the heightened vulnerability of domestic poultry environments to heavy metal pollution. This contamination likely results from industrial pollutants, urban runoff, or farming practices, leading to potential health risks for consumers through egg consumption (37).

These findings underscore the importance of targeted measures to manage heavy metal contamination in poultry products, particularly in domestic environments where exposure levels may be higher. It is essential to monitor and regulate the sources of contamination, such as animal feed, water, and environmental pollutants, to mitigate the risk of metal exposure through poultry products. Improved understanding of contamination pathways and control mechanisms could aid in minimizing the entry of hazardous substances into the food chain, supporting food safety and public health initiatives. Further research is recommended to explore the contamination patterns and preventive measures that can limit the risk posed by heavy metals in poultry products.

CONCLUSION

Heavy metals pose significant health risks, especially to vulnerable groups like children and the elderly, who may be more susceptible to contamination from dietary sources such as eggs. Elevated heavy metal levels in eggs are more common in developing countries, largely due to urbanization, industrial activities, and the absence of stringent regulatory measures, while industrialized nations generally exhibit lower contamination levels owing to stronger governance and enforcement. These findings underscore the need for regulatory agencies to enhance the clarity and enforcement of allowable limits for heavy metals in eggs, particularly for lead, cadmium, and copper. Establishing more rigorous monitoring and control systems can help mitigate exposure risks, thus supporting public health and fostering a safer food supply.

REFERENCES

1. Chowdhury MAZ, Abir M, Nesha M, Fardous Z, Rahman H, Bari ML. Assessment of toxic heavy metals and trace elements in poultry feeds, consumer chickens and eggs in Bangladesh. *Asian-Australasian Journal of Bioscience and Biotechnology*. 2021;6(3):128-41.
2. Malbe M, Otstavel T, Kodis I, Viitak A. Content of selected micro and macro elements in dairy cows' milk in Estonia. 2010.

3. Mottet A, Tempio G. Global poultry production: current state and future outlook and challenges. *World's poultry science journal*. 2017;73(2):245-56.
4. Jabeen S, Jamil I, Parveen K, Mansab S, Hussain M, Hussain S. Quantification of toxic metals in chicken egg and chicken feed via SOM-artificial neural network. *Environmental Monitoring and Assessment*. 2024;196(2):197.
5. Magdelaine P. Egg and egg product production and consumption in Europe and the rest of the world. *Improving the safety and quality of eggs and egg products*; Elsevier; 2011. p. 3-16.
6. Hossain A, Ahmed MW, Rabin MH, Kaium A, Razzaque MA, Zamil SS. Heavy metal quantification in chicken meat and egg: An emerging food safety concern. *Journal of Food Composition and Analysis*. 2024;126:105876.
7. Samad A, Roy D, Hasan MM, Ahmed KS, Sarker S, Hossain MM, et al. Intake of toxic metals through dietary eggs consumption and its potential health risk assessment on the peoples of the capital city Dhaka, Bangladesh. *Arabian Journal of Chemistry*. 2023;16(10):105104.
8. Gumpu MB, Sethuraman S, Krishnan UM, Rayappan JBB. A review on detection of heavy metal ions in water—an electrochemical approach. *Sensors and actuators B: chemical*. 2015;213:515-33.
9. Amir Ismail AI, Muhammad Riaz MR, Saeed Akhtar SA, Tariq Ismail TI, Mamoona Amir MA, Muhammad Zafar-ul-Hye MZ-u-H. Heavy metals in vegetables and respective soils irrigated by canal, municipal waste and tube well waters. 2014.
10. Bagal-Kestwal D, Karve MS, Kakade B, Pillai VK. Invertase inhibition based electrochemical sensor for the detection of heavy metal ions in aqueous system: Application of ultra-microelectrode to enhance sucrose biosensor's sensitivity. *Biosensors and Bioelectronics*. 2008;24(4):657-64.
11. Kanumakala S, Boneh A, Zacharin M. Pamidronate treatment improves bone mineral density in children with Menkes disease. *Journal of inherited metabolic disease*. 2002;25(5):391-8.
12. Guerrini A, Roncada P, Al-Qudah KM, Isani G, Pacicco F, Peloso M, et al. Content of Toxic Elements (Arsenic, Cadmium, Mercury, Lead) in Eggs from an Ethically Managed Laying Hen Farm. *Animals*. 2024;14(7):1133.
13. Raikwar MK, Kumar P, Singh M, Singh A. Toxic effect of heavy metals in livestock health. *Veterinary world*. 2008;1(1):28.
14. Aftab T, Shafiq T, Khan B, Chaudhry MN. Physicochemical properties, contamination and suitability of canal water for irrigation, Lahore branch Pakistan. *Pakistan journal of analytical & environmental chemistry*. 2011;12(1 & 2):7.
15. Aendo P, Mingkhwan R, Senachai K, Pinniam N, Sonthong K, Tulayakul P. Heavy metal contamination in eggs on poultry farms and ecological risk assessment around a gold mine area in northern Thailand. *Environmental Geochemistry and Health*. 2024;46(11):457.
16. Zain SM, Behkami S, Bakirdere S, Koki IB. Milk authentication and discrimination via metal content clustering—A case of comparing milk from Malaysia and selected countries of the world. *Food control*. 2016;66:306-14.
17. Korish MA, Attia YA. Evaluation of heavy metal content in feed, litter, meat, meat products, liver, and table eggs of chickens. *Animals*. 2020;10(4):727.
18. Chen CP, Zhang C-Y. Data-intensive applications, challenges, techniques and technologies: A survey on Big Data. *Information sciences*. 2014;275:314-47.
19. Humans IWGotEoCRt, Organization WH, Cancer IAfRo. Inorganic and organic lead compounds: IARC; 2006.
20. Biswas S. Birds as intrinsic bio-indicators for probing heavy metal contamination signatures in polluted Environmental matrices. *Heavy Metals-Recent Advances*: IntechOpen; 2023.
21. Tong S, Schirnding YEv, Prapamontol T. Environmental lead exposure: a public health problem of global dimensions. *Bulletin of the world health organization*. 2000;78(9):1068-77.
22. Tong ShiLu TS, Schirnding Yv, Prapamontol T. Environmental lead exposure: a public health problem of global dimensions. 2000.

23. Awuah KT, Finkelstein SH, Finkelstein FO. Quality of life of chronic kidney disease patients in developing countries. *Kidney international supplements*. 2013;3(2):227-9.
24. Roberts TL. Cadmium and phosphorous fertilizers: the issues and the science. *Procedia engineering*. 2014;83:52-9.
25. Anetor JI. Rising environmental cadmium levels in developing countries: threat to genome stability and health. *Nigerian journal of physiological sciences*. 2012;27(2):103-15.
26. Wang M, Chen Z, Song W, Hong D, Huang L, Li Y. A review on cadmium exposure in the population and intervention strategies against cadmium toxicity. *Bulletin of environmental contamination and toxicology*. 2021;106:65-74.
27. Bilandžić N, Đokić M, Sedak M, Solomun B, Varenina I, Knežević Z, et al. Trace element levels in raw milk from northern and southern regions of Croatia. *Food chemistry*. 2011;127(1):63-6.
28. Ullah AA, Afrin S, Hosen MM, Musarrat M, Ferdoushy T, Nahar Q, et al. Concentration, source identification, and potential human health risk assessment of heavy metals in chicken meat and egg in Bangladesh. *Environmental Science and Pollution Research*. 2022;1-12.
29. Eeva T, Lehikoinen E. Egg shell quality, clutch size and hatching success of the great tit (*Parus major*) and the pied flycatcher (*Ficedula hypoleuca*) in an air pollution gradient. *Oecologia*. 1995;102:312-23.
30. Jaffee S, Siegel P, Andrews C. Rapid agricultural supply chain risk assessment: A conceptual framework. *Agriculture and rural development discussion paper*. 2010;47(1):1-64.
31. Rokanuzzaman B, Salma U, Bristy NA, Kundu S, Alam SS, Khalil MI. Assessment of Heavy Metals and Trace Elements in Eggs and Eggshells of *Gallus gallus domesticus*, *Coturnix coturnix* and *Anas platyrhynchos* from Bangladesh. *Saudi J Biomed Res*. 2022;7(4):137-42.
32. Lehnher I. Methylmercury biogeochemistry: a review with special reference to Arctic aquatic ecosystems. *Environmental Reviews*. 2014;22(3):229-43.
33. Zahir F, Rizwi SJ, Haq SK, Khan RH. Low dose mercury toxicity and human health. *Environmental toxicology and pharmacology*. 2005;20(2):351-60.
34. Stankovic S, Stankovic AR. Bioindicators of toxic metals. *Green materials for energy, products and depollution*. 2013:151-228.
35. Wolfe MF, Schwarzbach S, Sulaiman RA. Effects of mercury on wildlife: a comprehensive review. *Environmental Toxicology and Chemistry: An International Journal*. 1998;17(2):146-60.
36. Boleli I, Morita V, Matos Jr J, Thimotheo M, Almeida V. Poultry egg incubation: integrating and optimizing production efficiency. *Brazilian Journal of Poultry Science*. 2016;18:1-16.
37. Fung F, Wang H-S, Menon S. Food safety in the 21st century. *Biomedical journal*. 2018;41(2):88-95.
38. Izah SC, Aigberua AO, Ogwu MC. Trace element composition of *Gallus gallus domesticus* eggs and health risks associated with their consumption in Port Harcourt, Nigeria. *Journal of Food Safety and Hygiene*. 2022.