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COMPARATIVE ASSESSMENT OF HEAVY METAL RESIDUES IN EDIBLE CHICKEN PRODUCTS EGGS, LIVER, AND GIZZARD FROM BROILER AND INDIGENOUS CHICKENS IN SELECTED REGIONS OF KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

The contamination of poultry food with heavy metals has become a critical food safety issue because of the health risks associated with these contaminants. The present study examined the levels of the metals Cu, Ni, Mn and Cr in eggs, liver and gizzard samples from both commercial broiler and indigenous (Desi) chickens in three environmentally different regions of Khyber Pakhtunkhwa (KP) province, Pakistan. Fifty-four samples were analyzed by using Atomic Absorption Spectrophotometry (AAS) and the potential human health risk was assessed by computing the estimated daily intake (EDI) and health risk index (HRI). The levels of the metals were significantly different in the various regions and tissues ($P < 0.05$), and the poultry products from the industrialized Hattar region had comparatively higher concentrations of the metals, especially Cu and Cr, whereas Ni contributed most to dietary exposure. The levels of metals in broiler liver and gizzard was generally higher compared to the indigenous chicken tissues. All concentrations detected were held to international limits, however, high Ni concentrations in some samples indicate that monitoring should continue. In summary the results show that present production of poultry products from the study area is safe for human consumption but points at the role of industrial activities on the accumulation of metals. Continuous monitoring of poultry products and production facilities is suggested to enhance food safety and safeguard public health.

1. Introduction

Poultry production is one of the rapidly expanding farm production industries in the world and contributes to food security by supplying cheap and good quality animal protein to the world. Poultry products (meat, eggs, liver and gizzard) are important sources of human nutrition since they contain significant amounts of proteins, essential amino acids, vitamins, minerals and polyunsaturated fatty acids (Perić et al., 2011). As serious demand for consumption of poultry products has grown, so has the intensity of poultry husbandry that may also contribute to the unintended contamination of the broilers by environmental pollutants, especially heavy metals (Rehman et al., 2012). Heavy metals are naturally occurring elements with a relatively high atomic weight, and a density value that is greater than 5 g/cm* (Henry and Miles, 2001). Some metals like copper (Cu), zinc (Zn), manganese (Mn), iron

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(Fe), chromium (Cr), and selenium (Se) are micronutrients that are needed for various physiological processes, enzymatic activities, gene regulation, and energy metabolism; however, an excess of these metals may be toxic and cause adverse health effects (Kennelly et al., 2018). Toxic metals like cadmium (Cd), lead (Pb) and arsenic (As) on the other hand have no known biological functions and are known to be harmful to animal and human health (Vukobratović et al., 2014). Heavy metal contamination of poultry products has become a serious food safety issue in the world. Heavy metals can be introduced into the poultry production chain through various sources, including environmental pollution, industrial emissions, contaminated water sources, agricultural practices, and the use of mineral supplements in the feed for the birds (Naveed et al., 2023). These metals can be deposited in various edible tissues and products in the animal such as eggs, liver, gizzard and muscle tissues which can then be incorporated in the diet of man (Burger, 2008). Eggs, especially, are known as excellent bioindicators as they easily accumulate trace metals from feed and environment (Korish et al., 2018). One of the main ways that humans are exposed to heavy metals is through eating food. Chronic exposure to high levels of these contaminants can lead to biomagnification and bioaccumulation up the food chain, triggering oxidative stress, cell dysfunction, neurological disorders, kidney and liver damage, reproductive difficulties, and/or an increased risk of carcinogenic and non-carcinogenic health effects (Tchounwou et al., 2012). Furthermore, the effects of interactions between essential and toxic metals on their absorption, metabolism and toxicity can impact the mineral homeostasis and physiological roles in living organisms (Pappas et al., 2010). Population growth and an increasing demand for inexpensive animal protein is expected to drive global poultry meat consumption to continue to rise (USDA, 2014). Thus, the safety and quality of poultry food products are a public health issue. While there are several studies examining the presence of heavy metals in poultry eggs or specific organs, few studies have examined multiple edible poultry products and/or in developing countries (Abdus et al., 2023). Urbanization, industrialization and intensification of agriculture in Pakistan can lead to high levels of environmental metals, which may lead to environmental contamination and high levels of environmental metals in poultry products. Hence, the current study was designed to assess the presence and concentration of heavy metals in edible poultry parts (eggs, liver and gizzard) of broiler chicken and indigenous (desi) chicken in some selected areas of District Haripur, Hattar and Abbottabad, K.P.Khanwah, Pakistan. Moreover, the study generated useful information regarding the food safety and health risks posed by the consumption of contaminated poultry products and thus can be utilized in evidence-based environmental monitoring, food quality assurance and public health protection strategies.

2. Materials and Methods

2.1 Study Area

This study has been carried out in three geo-locational areas of Khyber Pakhtunkhwa (KP) province, Pakistan, chosen to cover various environmental settings and pollutant sources. Hattar (Haripur District) is an industrial area with high anthropogenic influence, Haripur city is an urban /plain area with moderate anthropogenic influence and Abbottabad is a hilly area with less anthropogenic influence. The sites chosen allowed comparisons to be made of the accumulation of heavy metals in poultry products for different environmental exposure levels.

2.2 Sample Collection

During the study 54 poultry samples were collected from the poultry markets and retail chicken shops of Haripur city, Hattar and Abbottabad, which included 13 samples of chicken eggs, 11 liver and 30 gizzard. Samples were taken from: Commercial broiler chickens Indigenous (desi) chickens Liver and gizzard tissues were aseptically removed immediately after purchase for meat analysis. The fresh eggs were obtained from the same localities. Sterile

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polyethylene bags were used to collect the samples, which were labeled, refrigerated (4°C) and processed within 24 hours. A random sampling method was used to avoid sampling bias.

2.3 Sample Preparation

To prevent contamination from external metals, all laboratory glassware, crucibles and digestion vessels were treated by dipping in 10% nitric acid (HNO_3) for overnight and then cleaned with deionized water and air dried.

2.3.1 Washing

The livers and gizzards were thoroughly washed with deionized water to remove any blood, dust and extraneous material clinging to the organs. Egg shells were rinsed in distilled water and dried, then the contents carefully separated and analyzed.

2.3.2 Drying

Each edible sample weighing about 20-30g was then transferred to a labeled Petri dish and dried in a hot-air oven at 65°C to a constant weight.

2.3.3 Grinding

Dried tissues and egg samples were separately ground to a fine powder in a laboratory grinding machine. The powdered samples were packed in the air tight polyethylene containers for digestion.

2.4 Dry Ash Digestion

The dry ashing method was used for the heavy metal determination. About 2.0 g of the homogenized sample was placed in accurate acid-washed porcelain crucibles. The samples were initially pre-heated on the hot plate at 280°C for 30 minutes to eliminate any water and Volatile Organic Compounds (VOCs) that may be present. The crucibles were then placed in a muffle furnace and ashed at 350°C for 5 h to get light grey ash. The ash was subsequently cooled to room temperature in a desiccator and then digested with acid.

2.5 Acid Digestion

The ash was dissolved in 65% analytical grade nitric acid (HNO_3) and then diluted with deionized water. The solution was gently stirred until complete dissolution of the ash. The digested solution was filtered into acid washed volumetric flasks with Whatman No. 42 filter paper and the volume was adjusted with deionized water. The clear solution was kept in plastic tubes at 4℃ until instrumental analysis.

2.6 Heavy Metal Determination

The concentrations of Copper (Cu), Chromium (Cr), Nickel (Ni) and Manganese (Mn) were measured by Atomic Absorption Spectrophotometry (AAS) in the Technology Laboratory, COMSATS University Islamabad, Abbottabad Campus. Prior to the analysis of samples, calibration curves were prepared with certified standard metal solutions. Triplicate analysis was performed for each sample and the average results were used for statistical analysis. Concentrations of metals were reported as mg kg-1 wet weight (ppm). The services are subject to Quality Assurance and Quality Control (QA/QC) procedures. To ensure the accuracy, precision and reliability of the results, quality assurance and quality control (QA/QC) procedures were performed throughout the analytical process. To reduce potential contamination, all glassware, porcelain crucibles and digestion vessels were cleaned by soaking in 10% nitric acid and then rinsed with deionized water before use. All sample preparation and dilutions were carried out using analytical reagent grade chemicals and high purity deionized water. Procedural blanks were added in each digestion batch to monitor background contamination and to assure the integrity of the digestion process. Calibration curves were prepared using certified standard solutions before each analytical run and the analytical accuracy of the instrument was periodically checked with standard reference solutions. In addition, all samples were analysed three times and the mean values used for further statistical analysis to increase the accuracy and repeatability of the analysis.

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2.7 Statistical Analysis

Values are mean ± SE. Graphpad Prism software was used for statistical analysis. The differences of heavy metal concentration between regions and sample types of poultry were analysed by one-way ANOVA. Tukey's multiple comparison test was conducted when there were significant differences. The differences were considered statistically significant when $P < 0.05$.

3. Results

Heavy metals concentration in chicken eggs.

The result showed in (Figure.1 and Table1. Supplementary) the Means, Maximum and minimum level of heavy metals concertation in selected chicken eggs from various breads. The highest cupper concertation in ascending order is Desi Lowa Blue Yolk 1 (Hattar) at 0.545 mg/L,> Catalana Yolk 1 (Hattar) and Desi Lowa blue White 1 (Hattar), both exceeding 0.54 mg/L respectively. Where is lowest Cu metals concentration recorded in Catalana White 1 (Haripur) at 0.392 mg/L. The maximum and minimum Ni level observed in Catalana White 1 (Haripur) at 2.483 mg/L> Desi Lowa blue White 1 (Hattar) and Desi Lowa Blue Yolk 1 (Haripur) also had high Ni levels (above 1.2 mg/L). On the other hand, the lowest value was only 0.004 mg/L in Broiler Yolk (Abbottabad).

The highest Mn metals concentration in ascending order found in Desi Lowa blue White 1, Catalana and Broiler eggs were (Hattar) at 0.063 mg/L> 0.04 mg/L >0.002 > 0.015 mg/L. Where the maximum Cr concentration were 4.106 mg/L> 4.0 mg/L>1.184 mg/L >1.347 mg/L in Desi Lowa blue White 1 (Hattar), Broiler Yolk, Catalana White, and Desi Lowa Blue respectively.

Over all Cu metals concentration was found higher in Desi eggs (Hattar) region, lowest in Catalana (Haripur). Where Ni, level are Highest in Catalana White (Haripur), lowest in Broiler (Abbottabad). The Mn concentration level was slightly high in Hattar. Where Cr is dominates in Hattar, particularly Desi Lowa blue White 1 chicken eggs. The results showed moderate evidence of group differences, with some being significant at $P < 0.01$ (***) and others at $P < 0.05$ (*). $P > 0.05$ (ns) was found for a small number of comparisons, indicating that there was no significant change for those particular comparisons.

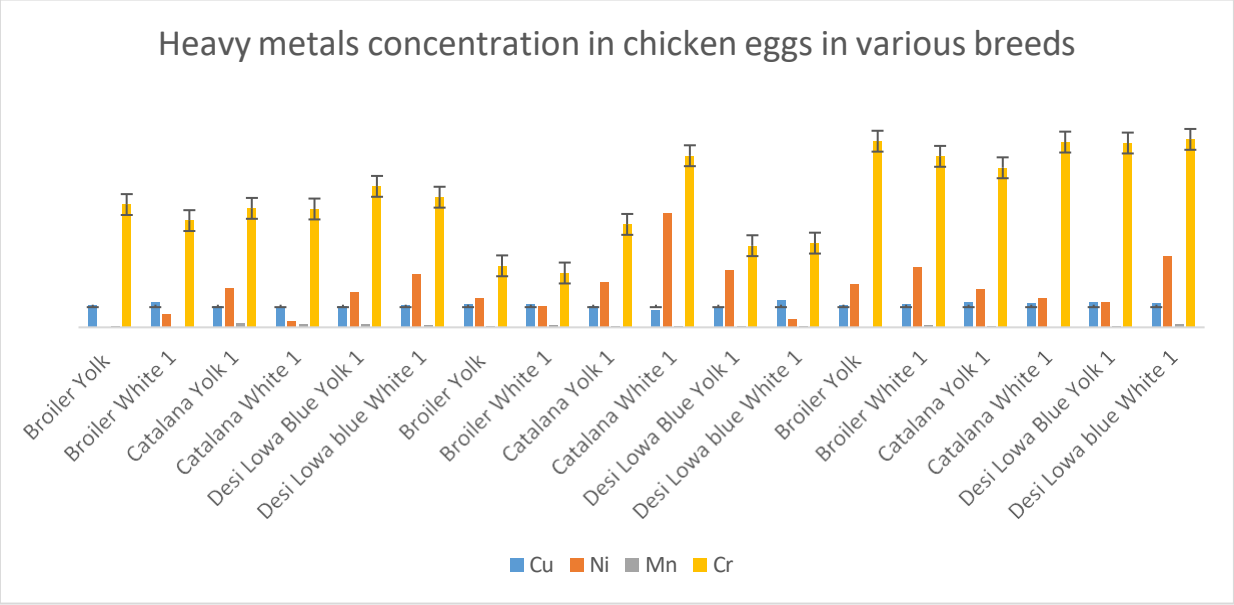


Figure.1 heavy metals concentration in chicken eggs.

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Heavy metals concentration in chicken Tissues.
In Figure.2 showed that highest concentration (maximum,minmum and means) were found in Abbottabad Cu in order from highest to lowest, broiler gizzard >Desiliver >Broiler liver and NI metals concentration noted in Broiler> Broiler gizzard > Desi liver and Desi gizzard. where is Mn concentration in order were Broiler liver>broiler gizzard> desi Liver > broiler gizzard. Cr metals concentration order is broiler liver >desi liver > desi gizzard >broiler gizzard in selected region in various chicken parts Broiler gizzard > Desi gizzard > Desi liver > Broiler liver Cu are found with high concentration.

Where the Cr concentration order found very low. In district Haripur the maximum concentration of Cu metals are found in order broiler liver >Broiler gizzard >Desi liver. While Ni metals concentration are Broiler liver>Broiler gizzard>Desiliver. Mn Broiler liver > Desi gizzard > Desi liver > Broiler gizzard and Cr Broiler liver > Broiler gizzard > Desi gizzard > Desi liver.

Where is the individual high concentration of Cu 5.785(Broiler liver) to 5.085(Broiler gizzard), 5.385 (Desi1 liver) and 5.885(Desi1gizzard), 5.085(Desi 2liver) and 5.585 (Desi2Gizzard) mg/ml respectivlly.Where is NI 0.119 (Broiler 1gizzard), 0.316 (Broiler 2 liver), 0.192 mg/ml and Mn 0.075, Desi 1 gizzard 0.132,0.08 (Broiler liver) /mg/ml. Further, examine the levels of heavy metals in different chicken we used a two way ANOVA. Whether the observed variations in metal concentrations are statistically significant is indicated by the p-values that are obtained. In our findings: $p < 0.001$ (*):** this suggests that there is a very substantial difference between the groups. This category includes almost all comparisons, indicating a high probability that variations in metal concentrations are actual and significant rather than the result of chance. $p < 0.05$ (*).

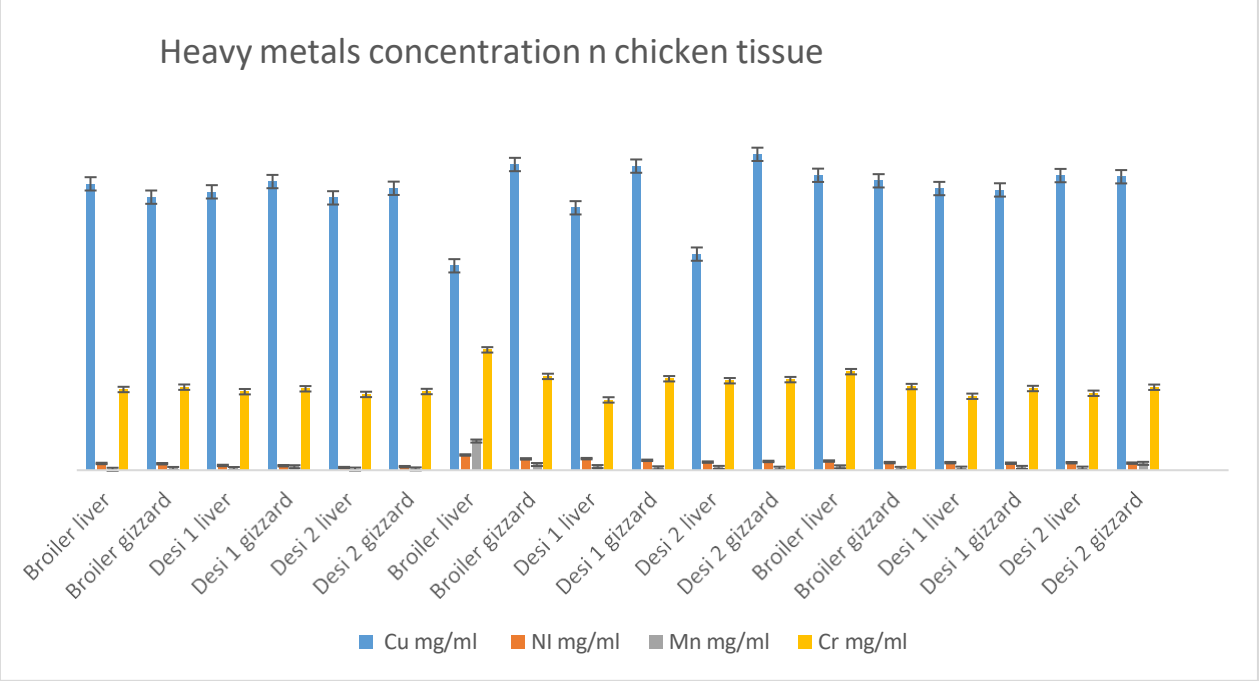


Figure.2 Heavy metals concentration in chicken Tissues.

Estimated daily Intake of metals (EDIM)
The estimated daily intake of the heavy metals compared with the RDA are presented in Table 3 Due to abnormally high amounts of nickel and chromium, Haripur's Desi Lowa Blue White eggs had the highest health risk index (HRI = 2.02). Although Hattar's HRI (0.124) in Desi Lowa Blue Yolk was noteworthy, it was still below Haripur's

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essential standards. All samples from Abbottabad exhibited relatively low HRI values, suggesting that metal ingestion poses little danger to health. All districts' broiler white samples frequently had lower HRI because of lower toxicity thresholds but higher DIM values for chromium. The findings indicate that different chicken parts from Abbottabad, Hattar, and Haripur had different daily intake (DIM) and health risk index (HRI) values of heavy metals (Cu, Mn, Ni, and Cr). Due to higher Ni levels, Desi 2 gizzards from all regions—particularly Hattar and Haripur—recorded the highest DIM. In general, the livers of broiler and Desi chickens had reduced HRI values, suggesting little health risk. Samples from Abbottabad had a moderate DIM but stayed within acceptable HRI ranges. In all samples, Ni was the metal that had the biggest impact on the health index.

Table.3. Daily intake of metals mg/kg/person/day and health risk index values

Region	Chicken tissue		Cu mg/ml	Mn mg/ml	NI mg/ml	Cr mg/ml	SUM OF DIM/HRI	
Abbottabad	Broiler liver	DIM	0.00043	0.02623	0.00911	0.000993	**3.68E-02**	
		HRI	0.000091	0.00011	0.0076	0.00551	1.33E-02	
	Broiler gizzard	DIM	0.00078	0.00058	0.00074	0.0978	**9.99E-02**	
		HRI	0.00034	0.00034	0.0012	0.00044	2.32E-03	
	Desi 1 liver	DIM	0.00043	0.00033	0.0031	0.00043	**4.29E-03**	
		HRI	0.00012	0.00042	0.00281	0.00002	3.37E-03	
	Desi 1 gizzard	DIM	0.0063	0.0003	0.0021	0.0063	**1.50E-02**	
		HRI	0.00056	0.00006	0.0656	0.00046	6.67E-02	
	Desi 2 liver	DIM	0.00098	0.00088	0.00021	0.00098	**3.05E-03**	
		HRI	0.00032	0.00012	0.00197	0.00032	2.73E-03	
	Desi 2 gizzard	DIM	1.0002	1.0012	0.00021	1.0002	**3.00E+00**	
		HRI	0.00031	0.00021	0.00011	0.00001	6.40E-04	
Hattar	Broiler liver	DIM	0.000321	0.000321	0.6276	0.00043	**6.29E-01**	
		HRI	0.0001	0.0011	0.00004	0.000001	1.24E-03	
	Broiler gizzard	DIM	0.66666	0.66676	0.0072	0.00078	**1.34E+00**	
		HRI	0.0004	0.00004	0.0001	0.00034	8.80E-04	
	Desi 1 liver	DIM	0.0032	0.0092	0.00081	0.00093	**1.41E-02**	
		HRI	0.0001	0.0001	0.00021	0.00012	5.30E-04	
	Desi 1 gizzard	DIM	0.00091	0.00081	0.0056	0.0063	**1.36E-02**	
		HRI	0.0021	0.0021	0.00721	0.00056	1.20E-02	
	Desi 2 liver	DIM	0.0056	0.0056	0.0097	0.00098	**2.19E-02**	
		HRI	0.00321	0.00721	0.0021	0.00032	1.28E-02	
	Desi 2 gizzard	DIM	0.0097	0.0097	1.0009	1.0002	**2.02E+00**	
		HRI	0.0001	0.0021	0.4571	0.00031	4.60E-01	
Haripur	Broiler liver	DIM	0.0002	0.0002	0.00023	0.0002	**8.30E-04**	
		HRI	0.001	0.0001	0.000011	0.001	2.11E-03	
	Broiler gizzard	DIM	0.00311	0.00811	0.00058	0.00311	**1.49E-02**	
		HRI	0.00001	0.00001	0.00034	0.00001	3.70E-04	

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	Desi 1 liver	DIM	0.0076	0.0076	0.00033	0.00976	**2.53E-02**	
		HRI	0.0066	0.0066	0.00042	0.0366	5.02E-02	
	Desi 1 gizzard	DIM	0.00045	0.00045	0.0003	0.00245	**3.65E-03**	
		HRI	0.0011	0.0011	0.00006	0.0011	3.36E-03	
	Desi 2 liver	DIM	0.0309	0.0409	0.00088	0.0509	**1.24E-01**	
		HRI	0.0041	0.0001	0.00012	0.0001	4.42E-03	
	Desi 2 gizzard	DIM	0.0021	0.0001	1.0012	0.0121	**1.02E+00**	
		HRI	0.0011	0.0001	0.00021	0.0061	7.51E-03	
	Broiler gizzard	DIM	0.00311	0.00811	0.00058	0.00311	**1.49E-02**	
		HRI	0.00001	0.00001	0.00034	0.00001	3.70E-04	
	Desi 1 liver	DIM	0.0076	0.0076	0.00033	0.00976	**2.53E-02**	
		HRI	0.0066	0.0066	0.00042	0.0366	5.02E-02	
	Desi 1 gizzard	DIM	0.00045	0.00045	0.0003	0.00245	**3.65E-03**	
		HRI	0.0011	0.0011	0.00006	0.0011	3.36E-03	
	Desi 2 liver	DIM	0.0309	0.0409	0.00088	0.0509	**1.24E-01**	
		HRI	0.0041	0.0001	0.00012	0.0001	4.42E-03	
	Desi 2 gizzard	DIM	0.0021	0.0001	1.0012	0.0121	**1.02E+00**	
		HRI	0.0011	0.0001	0.00021	0.0061	7.51E-03	

4. Discussion

The objective of the current study was to assess the levels of specific heavy metals (Cu, Ni, Mn, and Cr) in chicken eggs, liver, and gizzard from different breeds and geographical areas, such as Abbottabad, Haripur, and Hattar (an industrial zone). The results demonstrate that different egg types have varying amounts of these metals, with some breeds and geographical areas exhibiting comparatively high levels. All values, however, are within the acceptable ranges suggested by international food safety authorities (FAO/WHO, 2011). At 0.545 mg/L, Desi Lowa Blue Yolk 1 (Hattar) had the highest copper (Cu) values, followed by Catalana and Desi Lowa Blue White 1, all of which had levels above 0.54 mg/L.Catalana White 1 (Haripur) has the lowest Cu concentration, measuring 0.392 mg/L. Elevated copper concentrations may be caused by environmental exposure, especially in industrial areas like Hattar, even though these levels are within safe consumption limits (Ali et al., 2020). Even at tolerable levels, prolonged copper consumption can cause bioaccumulation and aggravate liver or renal disease (EFSA, 2015).

Levels of nickel (Ni) varied more sharply. Desi Lowa Blue White 1 and Desi Lowa Blue Yolk 1 both had Ni concentrations above 1.2 mg/L, while Catalana White 1 (Haripur) had the highest concentration at 2.483 mg/L. The lowest concentration was found in Broiler Yolk (Abbottabad) at only 0.004 mg/L. Although Ni is an essential trace element, excessive intake is associated with genotoxic, mutagenic, and carcinogenic effects (ATSDR, 2005). The increased Ni levels in some samples may reflect anthropogenic activities or feed contamination. The Hattar region had somewhat higher manganese (Mn) levels; Desi Lowa Blue White 1, Catalana, and Broiler eggs had 0.063 mg/L, 0.04 mg/L, and 0.015 mg/L, respectively. The FAO/WHO acceptable limits were met by all concentrations. Although manganese is necessary, excessive amounts can harm the reproductive system and brain system (WHO, 2004).

Hattar's proximity to industrial activity, which are recognized sources of Mn emissions, may be Eggs from the Hattar region have much higher quantities of chromium (Cr). Desi Lowa Blue

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White 1 (Hattar) had the highest Cr concentration, 4.106 mg/L, followed by Broiler Yolk, Catalana White, and Desi Lowa Blue, which had concentrations ranging from 1.184 to 4.0 mg/L.

Elevated Cr levels, particularly in its hexavalent form, are associated with serious health hazards, such as cancer and respiratory ailments, even though they are within acceptable bounds (USEPA, 1998). Because of the region's industrial history, the Desi eggs from Hattar generally had greater concentrations of Cu and Cr. On the other hand, broiler eggs from Abbottabad consistently had the lowest quantities of heavy metals, indicating comparatively low levels of environmental contamination, while Catalana eggs from Haripur displayed higher Ni levels. The levels of the heavy metals copper (Cu), nickel (Ni), manganese (Mn), and chromium (Cr) in the liver and gizzard of broiler and Desi chickens from the districts of Abbottabad and Haripur were assessed in this study. The results showed that although there were differences in the amounts of heavy metals between the samples, all quantities were within the acceptable ranges suggested by international health organizations (Rehman et al., 2012).

The broiler gizzard in Abbottabad had the greatest concentration of copper (5.682 mg/ml), followed by the broiler liver and desi liver. The concentration of Ni was higher in the gizzard (0.192 mg/ml) and liver (0.316 mg/ml) of broilers. Mn levels were somewhat higher in the desi gizzard (0.075 mg/ml) and broiler liver (0.132 mg/ml).In line with the findings of Rehman et al. (2012), who documented tissue-specific metal accumulation with the liver and gizzard serving as important bioaccumulation sites, Cr was detected in comparatively lower concentrations in all samples. Similar patterns were noted at Haripur. The highest concentration of copper (5.776 mg/ml) was found in the liver of broilers, followed by the gizzard and desi liver. In addition, broiler organs had higher amounts of Ni and Mn than desi tissues. In every tissue analyzed, Cr continued to be the least accumulating metal. These results are consistent with other research showing that feed composition, environmental exposure, and the metabolic potential of various chicken breeds all affect the accumulation of metals including Cu, Ni, and Mn (Mccrory et al., 2005; Ayar et al., 2009). Even though these metals are within safe limits, if they are not routinely checked, their bioaccumulation over time may provide health risks. Human hepatic, renal, and neurological diseases are among the serious physiological alterations that can result from chronic exposure, even at low concentrations (Daniel & Edward, 1995; Cunningham & Saigo, 1997). Thus, to protect the public's health, poultry feed and environmental conditions must be systematically monitored and regulated. Furthermore, as eggs and chicken meat make up a large portion of human nutrition, Sparks and Surai (2013) underlined the significance of comprehending metal deposits in these foods. Research on trace elements in foods obtained from animals (Pappas et al., 2006; Baykov et al., 1996) emphasizes the importance of taking into account both genetic and environmental factors that affect metal deposition.

5. Conclusion

Meat and chicken eggs from Abbottabad and Haripur and Hattar had heavy metal concentrations that were within acceptable dietary bounds. Comparing broiler organs to Desi hens, the concentrations were comparatively greater. A decreased dietary risk was indicated by the limited contamination seen in egg samples. To guarantee food safety and stop future contamination, routine observation is advised. Conclusion, regional industrialization, particularly in Hattar, may have contributed to the comparatively greater metal concentration even if all detected values of Cu, Ni, Mn, and Cr were within acceptable safety tolerances. To avoid further accumulation and guarantee food safety, this calls for ongoing monitoring and risk assessments

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