Determination of Heavy Metals in Domestic and Broiler Chickens Meat in Quetta City

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Abstract
Poultry meat is a high-protein source that is consumed all over the world. This source is tainted not only by viruses but also by environmental contaminants, including HM. This study aimed to detect HM in the tissues (liver, gizzard, muscles, heart and kidney) of BC and DC in Quetta city, Balochistan, Pakistan for the first time, using AAF. Samples were collected from different regions of the district, and prepared for analysis. The measured Ct (mg/L) of detected HM like Pb (0.30-0.83), Co (0.18-0.35), Mn (0.10-0.21), Fe (0.32-12.70) and Cu (3.79-4.51) varied across different organs in analyzed BC and DC samples. Cu, Mn, Co and Fe content was within the range of acceptable values established by WHO and ANZFA. However, Ct of Pb for BC and DC exceeded WHO's (0.2 mg/L) and EU's (0.1 mg/L) permissible limits. Therefore, more in depth research studies on poultry feed should be carried out, for its improvement and safety.

Keywords: AAS; BC; DC; HM.

Introduction•

Anthropogenic activities in agriculture and industry are causing HM pollution in food and feed every day. Due to contaminated feed, drinking water and litter, poultry is exposed to a vast array of metals, which can lead to a reduction in the safety of these food products [1]. Different processes within industries lead to pollution entering the food chain and contaminating our food [2, 3]. Poultry meat provides essential amino acids, vitamins, minerals, important trace elements, antioxidants and protein to humans, particularly in developing countries [4]. Compared to other meat products, poultry meat is relatively cheap, available on large scale, and meets worldwide protein requirements. In addition to the livestock health, production and reproduction, poultry feed contamination with HM also affects the safety of its products [5]. In Pakistan, poultry farms play an

•The abbreviations list is in page 361.

important role in the country's economy and food supply. For many years, poultry industry was exempted of taxes, for its promotion [6]. Pakistan's poultry sector is expanding tremendously in bridging the gap between protein demand and supply. Due to the shortage and high prices of red beef, mutton and camel meat, poultry is the only affordable source of protein for people [7]. The poultry industry produces 25.8% of meat in Pakistan [8]. It is essential for human diets worldwide, as it globally tackles nutritional issues and provides adequate protein, Ca, vitamins and other nutrients [9].

Chicken meat may contain multiple HM such as As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn [10]. HM are transmitted into chicken's meat by different sources such as feed, drinking water, polluted slaughtering places and automobile exhausts [11]. Chicken feed includes fish, soybean, meat scrap, wheat, milk byproducts, oats, sunflower grain, liver food and corn. Poultry obtains protein, carbohydrates, minerals and vitamins from these feeds [12]. Poultry feeds such as leaves, seed meal, sorghum and grain byproducts contain amino acids and different minerals like Ca, Cu, Fe, K, Mg, Mo, Na, P, S and Zn [6].

Due to the worldwide high consumption of poultry as food supply, and to the complexities involved with its use in terms of exposure to HM, it is important to examine metals content in different tissues of most commonly consumed chickens. This current study aimed to assess HM in BC and DC randomly gathered from different regions in Quetta.

Methods and materials

Reagents

All employed chemicals and reagents were of analytical grade. Ultra-high pure deionized water was used for dilutions. A bath with 20% HCl was used for cleaning all plastic and glassware, followed by deionized water. A 10 mL mixture of concentrated HNO_3 and $HClO_4$ was used processing each sample in a 4:1 ratio. Throughout the analysis, a blank solution of 0.5 M HNO_3 was used. To prepare the standard solutions for different metals, 1000 mg/L stock solutions were used by serial dilution with 0.5 M HNO_3.

Samples collection and preparation

BC and DC organs were randomly collected in polyethylene bags from Quetta city. In the laboratory, the previous protocol was used with slight changes [13]. The obtained samples were washed with deionized water, to remove contaminant/blood particles The samples were cut into small pieces using a sterile scalpel knife, and dried in an oven, at a temperature of 120 ºC, before a steady weight was reached. Afterwards, a ceramic pestle and mortar were used to grind the samples into a fine powder, which were placed in polyethylene sacks. 1 g of each sample's dry powder was taken after weighing. It was then transferred to the beaker and processed with a mixture of acids. The solution was heated at 100 ºC, for around 20 min. Later, it was cooled and filtered using Whatman paper (No. 42), and diluted with UHP deionized water up to a final volume of 50 mL. The obtained solutions were stored at room temperature for further study.

Instrumentation and procedures

Several metals such as Co, Cu, Fe, Mn and Pb were evaluated in BC and DC samples. Co, Cu, Fe, Mn and Pb detection were performed by AAS (Thermo-Electron Corporation, S4 AA System, Ser No, GE711544, China).

Analytical validation parameters

The proposed methods were validated through LOD and LOQ values, according to eqs. (1) and (2), for calibration method [14]. The solution Ct was 1000 mg/L. Analytical curves were drawn for five solutions with Ct from 0.5 to 2.5 mg/L. Based on their average, LOD and LOQ values were determined.

$$LOD = 3 \times (\sigma/s) \quad (1)$$

$$LOQ = 10 \times (\sigma/s) \quad (2)$$

where σ is the response or y-intercepts SD and s is calibration slope.
Precision was expressed as %RSD, and accuracy assessment was determined using a reference standard with a Ct range from 0.5 to 2.5 mg/L.

Results and discussion

Poultry meat is used as a food source on a large scale in our region. It may be contaminated with HM from feed and other environmental sources. This study showed the presence of all HM detected in BC and DC, in Quetta. Fig. 1 depicts research methodology.

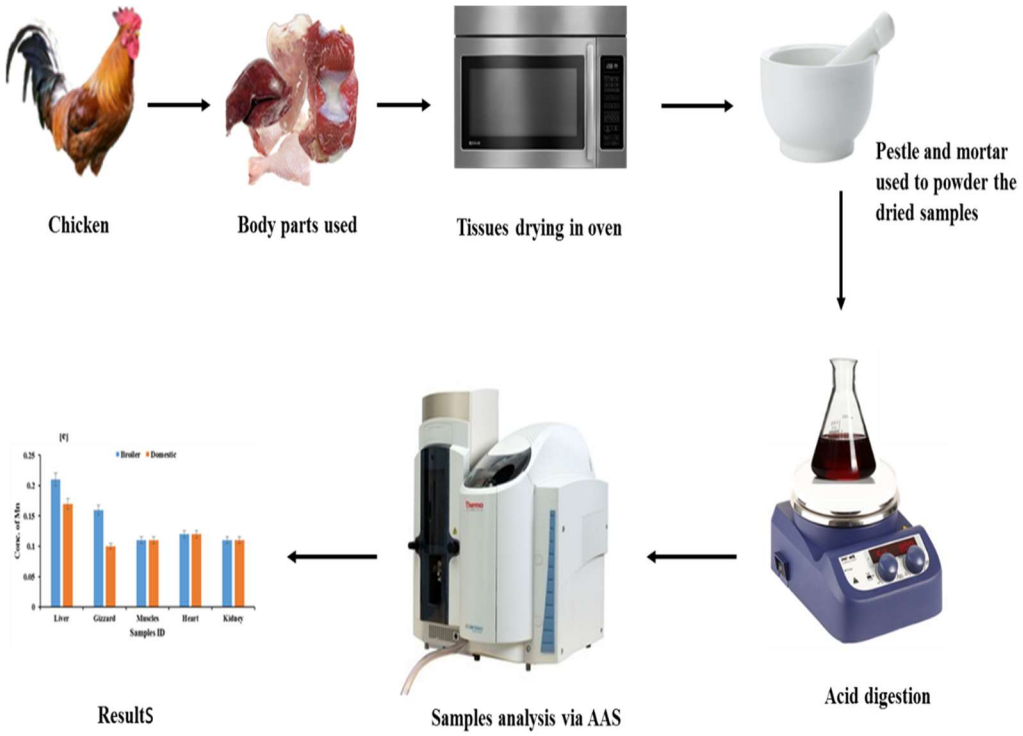

Figure 1: Schematic diagram of research methodology.

The results of detected HM accumulation in BC and DC are displayed in Tables 1 and 2, respectively.

Table 1: Measurement of HM Ct in different organs of BC.

Samples ID	HM (Mean ± SD*)				
	Pb (mg/L)	Co (mg/L)	Mn (mg/L)	Fe (mg/L)	Cu (mg/L)
Liver	0.41 ±0.05	0.33 ±0.11	0.21 ±0.01	6.51±0.44	4.03 ±0.32
Gizzard	0.45 ±0.02	0.35 ±0.35	0.16 ±0.04	2.42±0.44	4.39 ±0.43
Muscles	0.43 ±0.05	0.33 ±0.11	0.11 ±0.03	1.22±0.46	4.04 ±0.37
Heart	0.83 ±0.03	0.31 ±0.47	0.12 ±0.44	1.32±0.03	3.79 ±0.36
Kidney	0.68 ±0.06	0.31 ±0.47	0.11 ±0.03	4.43±0.37	4.22 ±0.43

Table 2: Measurement of HM Ct in different organs of DC.

Samples ID	HM (Mean ± SD*)				
	Pb (mg/L)	Co (mg/L)	Mn (mg/L)	Fe (mg/L)	Cu (mg/L)
Liver	0.30 ±0.46	0.25 ±0.03	0.17 ±0.05	9.26 ±0.47	4.18 ±0.45
Gizzard	0.25 ±0.32	0.21 ±0.01	0.10 ±0.01	4.21 ±0.42	4.32 0.48
Muscles	0.48 ±0.34	0.27 ±0.04	0.11 ±0.03	1.44 ±0.49	4.36 ±0.47
Heart	0.26 ±0.04	0.18 ±0.02	0.12 ±0.04	3.44 ±0.47	4.51 ±0.52
Kidney	0.28 ±0.06	0.19 ±0.03	0.11 ±0.03	12.70 ±0.56	3.85 ±0.34

Analytical performance, such as LOD, LOQ, precision and accuracy, was statistically assessed to optimize AAS parameters, and to evaluate the investigated procedure. LOD LOQ and RSD values on precision obtained for HM determined by AAS method are reported in Table 3. LOD and LOQ ranged from 0.05 to 0.80 mg/L and from 0.2 to 2.8 mg/L, respectively. In this study, processing efficiency was assessed under repeatability conditions, using RSD% calculated for all investigated HM. RSD% low values indicated high precision of the proposed method. In all cases, obtained R^2 was 0.99.

Table 3: Analytical evaluation parameters for different HM.

Metals	LOD (mg/L^{-1})	LOQ (m/gL^{-1})	RSD(%)	R^2
Co	0.06	0.2	1.7	0.9998
Cu	0.05	0.2	1.4	0.9999
Fe	0.8	2.8	3.6	0.9993
Mn	0.05	0.2	1.4	0.9999
Pb	0.07	0.2	1.9	0.9998

Ct for Pb in the heart, kidney, gizzard and liver of BC were found to be greater than those of DC. However, DC's muscles had higher Ct than that from BC (Fig. 1). The present analysis reported higher Ct of Pb in poultry than the allowable levels set by WHO and EU (0.2 and 0.1 mg/L, respectively). Vehicles' exhaust gases and wastewater used for irrigation purposes in Quetta can pollute poultry feed with Pb. High Ct of Pb can cause lethal diseases, such as cancer. The involved authorities must take very timely steps to ban the use of wastewater on irrigation.

Although at low Ct Fe plays a significant part in the health of human beings, at high ones, it can become toxic. In DC, the approximate Ct of Fe was higher than that of BC (Fig. 1). In this study, Ct for Fe were below the results of previous studies [15, 16]. WHO and FAO set no permissible limit of Fe. However, FAO and NRC recommend the limit of 40 mg/L for chickens' meat. The latest study

has shown that BC and DC's approximate Fe levels are lower than those specified by NRC. Consequently, they should not harm humans.

Co plays a significant role in producing vitamin B_{12} and assuring the proper functioning of human body. Co usually reaches the body via salt [17]. BC's organs indicated a greater Ct of Co than that of DC (Fig. 1), which might be due to the variation in its Ct on the feed given to the former. Co analysis showed higher Ct than previous researches [18, 19]. However, the present study concluded that Ct of Co was lower than WHO's standard value of 1.5 mg/L.

Cu is considered a critical component of numerous enzymes. It participates in bone formation, skeleton mineralization, and even preserves connective tissue integrity. DC's heart contained higher Ct of Cu than that of BC, but BC's kidney displayed higher levels of Cu than that of DC. However, no substantial variance in the Ct of Cu was observed among gizzards, livers and muscles of BC and DC (Fig. 1), although it was higher than the results of previous studies [20, 21]. Overall, the present research study concluded that the Ct of Cu was within the range of standard value.

Prolonged exposure to high levels of Mn may cause a severe disorder, and it can contribute to pneumonitis and lung cancer. BC's gizzard, liver and kidney showed higher levels of Mn than those of DC. However, no variation was detected in BC's and DC's hearts and muscles (Fig. 2a-e). This work demonstrated that Mn levels are equivalent to previous findings [22, 23]. Nevertheless, this current analysis indicated a lower Mn level than WHO's permissible limit (0.5 mg/L).

Fig. 2a-e show the distinction between BC's and DC's tissues, and Fig. 3a-e depict calibration curves.

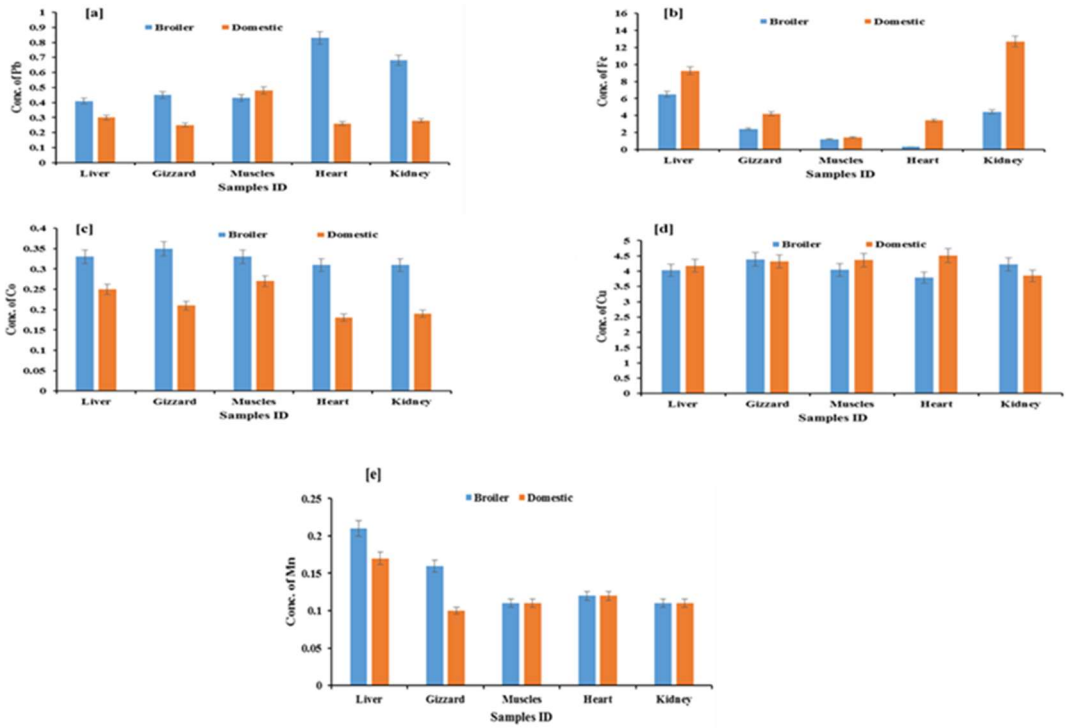

Figure 2: Ct of **(a)** Pb, **(b)** Fe, **(c)** Co, **(d)** Cu and **(e)** Mn in BC's and DC's different tissues (ppm).

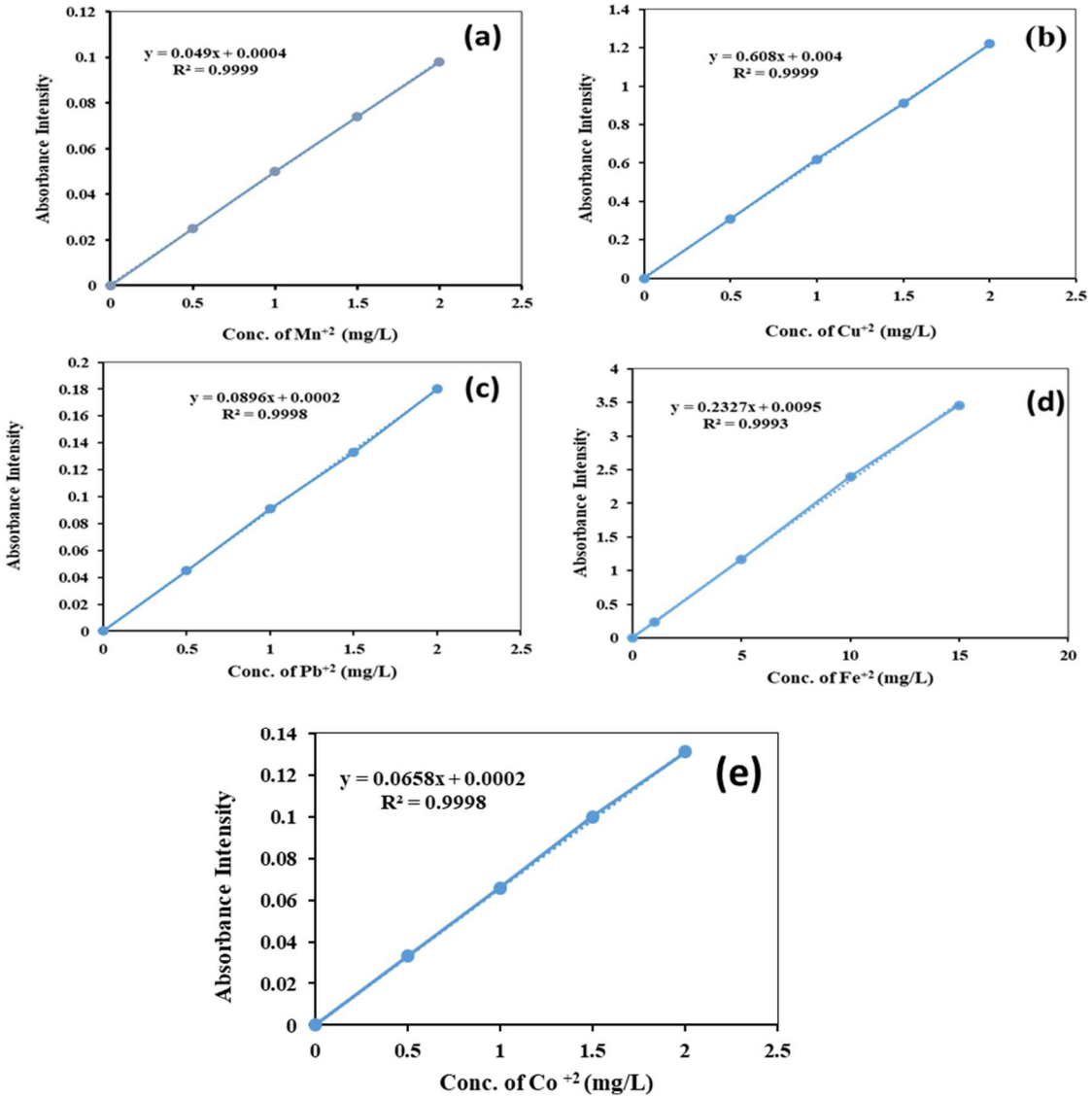

Figure 3: Calibration curves for- **(a)** Mn, **(b)** Cu, **(c)** Pb, **(d)** Fe and **(e)** Co.

Conclusion

The present study showed the presence of HM in BC and DC organs, which might be due to polluted environment and feed consumed by poultry. Ct of Fe for DC was higher than that of BC. However, Ct of Pb in BC organs was higher than that of DC, due to the various proportions of ingredients in poultry feed. DC's liver, kidney, muscles and heart had higher Ct of Cu and Mn than those of BC, while its Fe, Co, Cu and Mn were found within WHO's and ANZFA's permissible limits. However, Pb has exceeded WHO's permissible limit for BC and DC. On the basis of above detected HM, further research studies are needed on poultry feed, for health concerns.

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Authors' contributions

A. Hakeem: conducted experiments; gathered and analyzed data. **N. Khan**: conceived and designed research study. **A.-ur-R. Kakar**: provided chemicals and reagents. **Samiullah**: prepared the draft. **M. Asghar**: assisted on data interpretation. **G. M. Khan**: edited and reviewed the manuscript.

Abbreviations

AAF: atomic absorption spectrometer
ANZFA: Australia New Zealand Food Authority
BC: broiler chicken
Ct: concentration
DC: domestic chicken
FAO: Food and Agriculture Organization of the United Nations
HCl: hydrochloric acid
$HClO_4$: perchloric acid
HM: heavy metals
HNO_3: nitric acid
LOD: limit of detection
LOQ: limit of quantification
NRC: National Research Council
ppm: parts per million
R^2: coefficient of determination/relation
RSD: relative standard deviation
SD: relative standard deviation
UHP: ultra high-pressure
WHO: World Health Organization

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