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CONCENTRATIONS OF HEAVY METALS IN LIVER, MEAT AND BLOOD OF POULTRY CHICKEN *GALLUS DOMESTICUS* IN THREE SELECTED CITIES OF PAKISTAN

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ABSTRACT

Heavy metal contamination poses a serious threat to human health due its biomagnifications, bioaccumulation and toxicity. It has caused widespread concern about human health, and therefore, the scientists are focusing their study on the levels of concentration of these metals in food consumed by humans, so as to evaluate the risk associated with heavy metals exposure. In the present study, concentrations of heavy metals (Cadmium, Lead, Copper, Nickel and Zinc) were determined in Poultry chicken *Gallus domesticus* from some selected areas in the cities like Karachi, Hyderabad and Thatta all in the province of Sindh. A total of 135 poultry organs (liver and meat) and blood were randomly collected from chicken, aged 8-10 weeks old, weighing 1.2 to 1.5 kg. The samples were analyzed using Atomic Absorption Spectroscopy for detection of five heavy metals. All samples showed presence of Cadmium, Lead, Nickel, Zinc and Copper. The levels recorded in the samples were under the permissible limits recommended by the WHO, FAO and ANZFA, except that in the samples from Karachi, the levels of Ni and Pb exceeded the maximum permissible limits in liver, meat and blood. In the samples from Hyderabad, the levels of concentration of Ni and Pb in liver and meat, and only Ni in blood exceeded the permissible limits. In the samples from Thatta city, Ni and Pb in liver and meat, and only Pb in blood exceeded the permissible limits. The present study indicated that heavy metal contaminants come from unhygienic poultry feed material.

Keywords: Karachi, hyderabad, thatta, poultry chicken, heavy metals.

INTRODUCTION

Heavy metals including copper, cadmium, zinc, nickel and lead are abundantly found in the ecosystem and can be detected in small quantities in the fish and birds. The main route via which humans get exposed to these metals is through the ingestion of contaminated food, especially birds and fish. The ultimate source of all metallic elements present in the ecosystem is our earth's crust. In the environment nearly 40 elements are classified as metals. Some important molecules contain metals like copper, nickel, zinc, cobalt, manganese as their structural constituents. These trace elements also act as cofactors in number of enzymes catalyzing different metabolic processes, while excessive concentrations of these elements are toxic (Chang and Cockerham, 1994).

Metal contamination in food additives or land foodstuff has been noticed and concerned authorities (WHO) have proposed the highest permissible levels of metals contamination in foodstuff. These proposed permissible levels help the food industries in the selection of better quality raw material and use of equipment. According to the WHO experts, diet pattern should be thoroughly analyzed; so that, each of the products could be analyzed individually, for its individual contribution of metal concentration in total metal toxicity. The primary causes of accumulation of heavy metals in edible products are natural conditions, fertilizers and industrial pollution. Since the climatic conditions, such as soil texture and other concerned environmental agents, vary largely according to the geographical location, the amount of heavy metals also varies, from one place to other. The evaluation of exposure of the population to these contaminants can be achieved by analysis of market products.

Heavy metals taken in traces, bind with proteins and become nontoxic, but their high concentration, above the body tolerance level, results into severe pathological

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conditions. Their increased concentration reacts with important cellular components through, covalent and ionic bindings, resulting in damage to cell membrane and alteration of the normal cellular functions i.e. the enzymatic and the normal molecular systems of the cell. These metals alter the gene code by damaging the DNA structure (Bruins *et al*., 2000; Blasliak *et al*., 1999).

Heavy metals accumulate in the biological system and they affect the birds in different ways. Poultry is generally exposed to different heavy metals poisoning through contaminated water, poultry feed, sewerage water, industrial effluents and also through aerial sprays in poultry breeding areas. Another factor that contributes greatly to the contamination of meat is that it is sold, mostly, in open markets and sometimes also on the roadsides. This causes illness in human beings (Sattar and Chaudhary 1978; Khurshid and Qureshi, 1984). The infected birds may act as causative agents for many fatal diseases to human in Pakistan (Raja *et al*., 1996). Since heavy metals have a highly toxic nature, even at relatively minute concentrations, therefore it is of utmost importance, both for food safety and human health, to closely monitor their concentration in meat and meat products, so as to minimize the risk of heavy metal contamination (Santhi *et al*., 2008). The processing method can also give way to contamination of meat products via the raw materials, spices and water used also during the packaging process (Raikwar *et al*., 2008; Santhi *et al*., 2008).

Cadmium is a toxic metal. It is being used in corrosion prevention, polymer electronics and pigments since the 1960's (Qadir *et al*., 1999). Cadmium uptake in higher animals including human, occurs mostly by ingestion (Jinkins *et al*., 1980), whereas exposure is not significant via dermal contact. Foodstuffs are a common route of cadmium exposure since many plants and especially fish and crustaceans accumulate cadmium very efficiently. Cadmium is a classic cumulative poison that accumulates in the kidneys over a lifetime. Acute cadmium toxicity in humans occurs due to taking in considerably high concentrations from contaminated beverages and food. This causes local irritant effects on the gastrointestinal tract including nausea, vomiting, abdominal pain, a choking sensation and diarrhea. Pulmonary edema; resulting possibly in death, and chemical pneumonitis may occur as a result of cadmium fumes' inhalation. Chronic Cadmium exposure has adverse effects on the lungs, heart gonads, bones and especially the kidneys. Anemia may also result due to long-term exposure to cadmium (Wexler and Shayne, 1998a).

Lead is a highly toxic metal and non-essential metal present in all tissues and organs of mammals (Donald and Flegal, 1995). Lead and lead-containing compounds are commonly used in lead-acid storage batteries, sheet metal, piping, ammunition and paints. Lead exposure can occur via ingestion, inhalation and dermal contact. The most common route of exposure of the general population to lead is the ingestion of contaminated water and food. In the workplace, the most significant route of Lead exposure is inhalation (Wexler and Shayne, 1998b). Cigarette smoke also contains small amount of Lead (Stone and Dropper, 1996). Lead can be present in drinking water as a consequence of leaching from old pipes, faucets etc. In young children, Lead poisoning may occur due to ingestion of peeling and chipping lead-based paint in older homes. Lead affects many organs, physiological functions and cellular and molecular processes in the body. It impedes several cellular signaling processes, the activity of various enzymes and the action potentials in certain nerve cells. The functionality of the thyroid and the adrenal glands is also affected by Lead. The kidneys, the blood and the nervous system are the principle target organs. Reproductive effects; miscarriage, male infertility and neonatal morbidity and mortality, may also be seen. Impairment of the immune system may also occur with joint pains related to gout and cardiac fibrosis and myocarditis as consequences (Wexler and Shayne, 1998b).

Nickel is a widely distributed, 24th element in order of natural abundance, in the earth's crust (Kasprzak *et al*., 2003). Nickel and its compounds are abundantly used in nickel-cadmium batteries, certain glazes and pigments, plated coatings and as laboratory and industrial catalysts. Humans and animals may be exposed to nickel and nickel containing compounds through ingestion, inhalation and dermal contact. Smoke from the combustion of coal and petroleum products and emissions from certain industrial products are a cause of releasing nickel into the ambient air. Nickel exposure may result in adverse reactions at the sight of contact; gastrointestinal tract, skin or respiratory tract, or systemically; in kidneys, heart and blood. It may lead to pneumonia, pulmonary edema and death. Nickel exposure is also related with cancer development (Wexler and Shayne, 1998b).

Zinc is constituent of all cells and is an essential trace element, commonly ingested as a nutritional supplement. It acts as a co-factor for number of enzymes. Zinc is a necessary and useful element in human growth (Renfro *et al*., 1975). Its deficiency can affect humans and in extreme cases, hair loss, impaired growth, skin eruptions, short stature and delayed or impaired sexual maturity are the effects. Ulcerative colitis, anemia and chronic renal disease have also been associated with Zinc deficiencies (Wexler and Shayne, 1998c). The possible exposure pathways of Zinc are ingestion and inhalation. Most plants absorb Zinc readily therefore majority of foods are rich in Zinc (Alloway and Ayres, 1994). Zinc, when present in excessive amounts, interferes with Copper and Iron metabolism; the former causes copper-deficiency

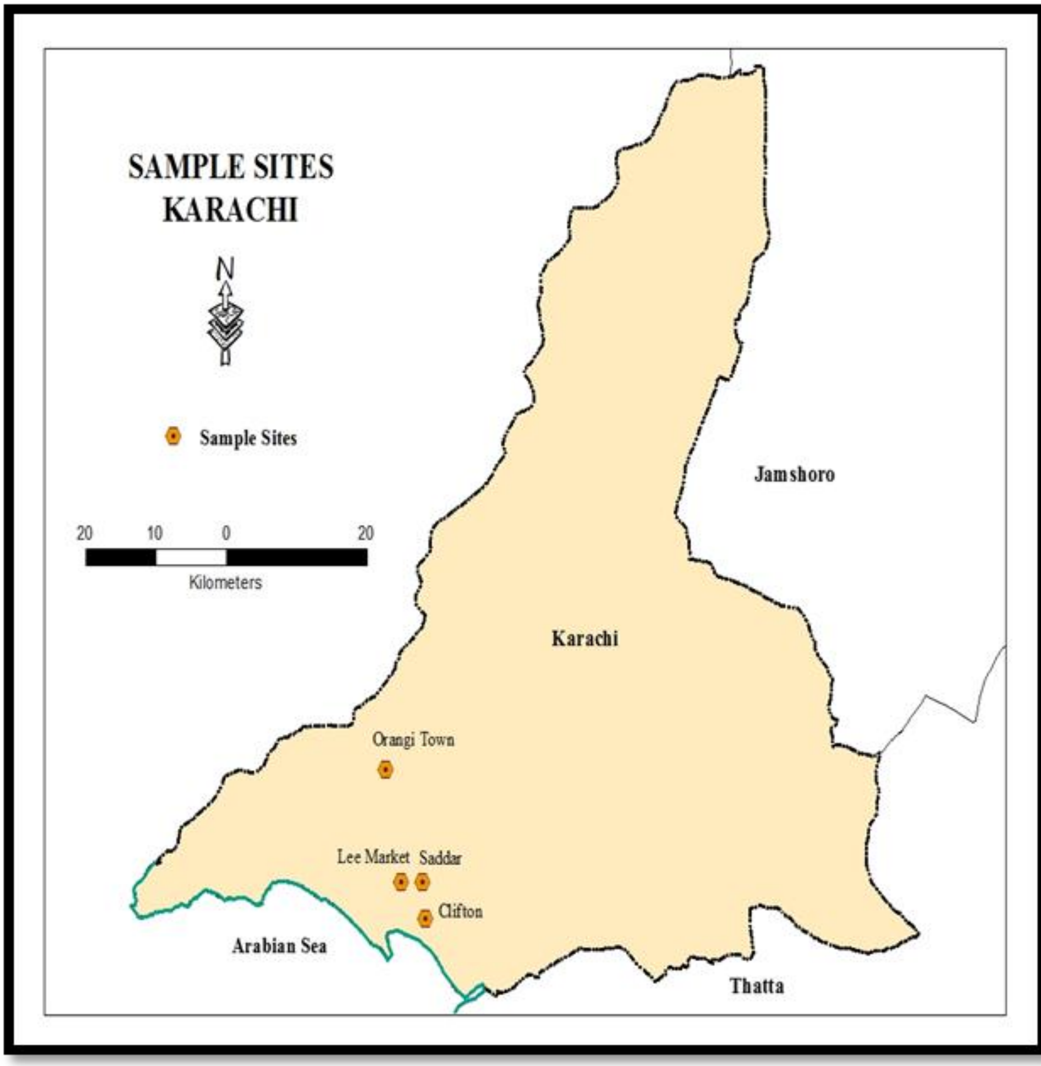

Fig. 1. Map of study areas of Karachi.

anemia. Zinc ingested in excessive amounts can cause cramps, nausea, vomiting and diarrhea. Inhalation of fumes of Zinc results in pulmonary edema and metal fume fever with symptoms including sweating, weakness and chills alternating with fever which can continue up to 24 to 48 hours. Liver damage, sometimes even fatal, can occur due to chronic inhalation of Zinc (Wexler and Shayne, 1998c).

Copper is an essential trace element found abundantly in nature. It is used as a component in a variety of alloys, as a constituent in paints and ceramic glazes and as an electrical conductor. Humans are usually exposed to Copper via ingestion e.g. water or food. Another source of this element is copper pipes through which drinking water is carried (Qadir *et al*., 1999). Excessive intake of Copper results in its accumulation in the liver. If excessive amounts of Copper salts are ingested, acute poisoning occurs with nonspecific toxic symptoms; nausea, vomiting and a metallic taste. Ulceration of the gastrointestinal tract can also occur. Other severe symptoms can also occur including jaundice, hypotension, coma and death. Copper toxicity can sometimes result in an inability to urinate while liver necrosis has also been observed. Presence of excess Copper inhibits the amino acid transferases, also causing lipid peroxidation. Copper is also associated with two inborn errors of metabolism; one is Menke's disease or Menke's kinky hair disease and the other is Wilson's disease or hepatolenticular degeneration (Wexler and Shayne, 1998a).

Heavy metal contamination is considered a major threat to human health worldwide. In Pakistan we are facing heavy metals pollution in our food products. The objective of the present study was to determine the levels of heavy metals (Cadmium, Lead, Nickel, Zinc and Copper) in the liver, meat and blood of poultry chicken *Gallus domesticus*

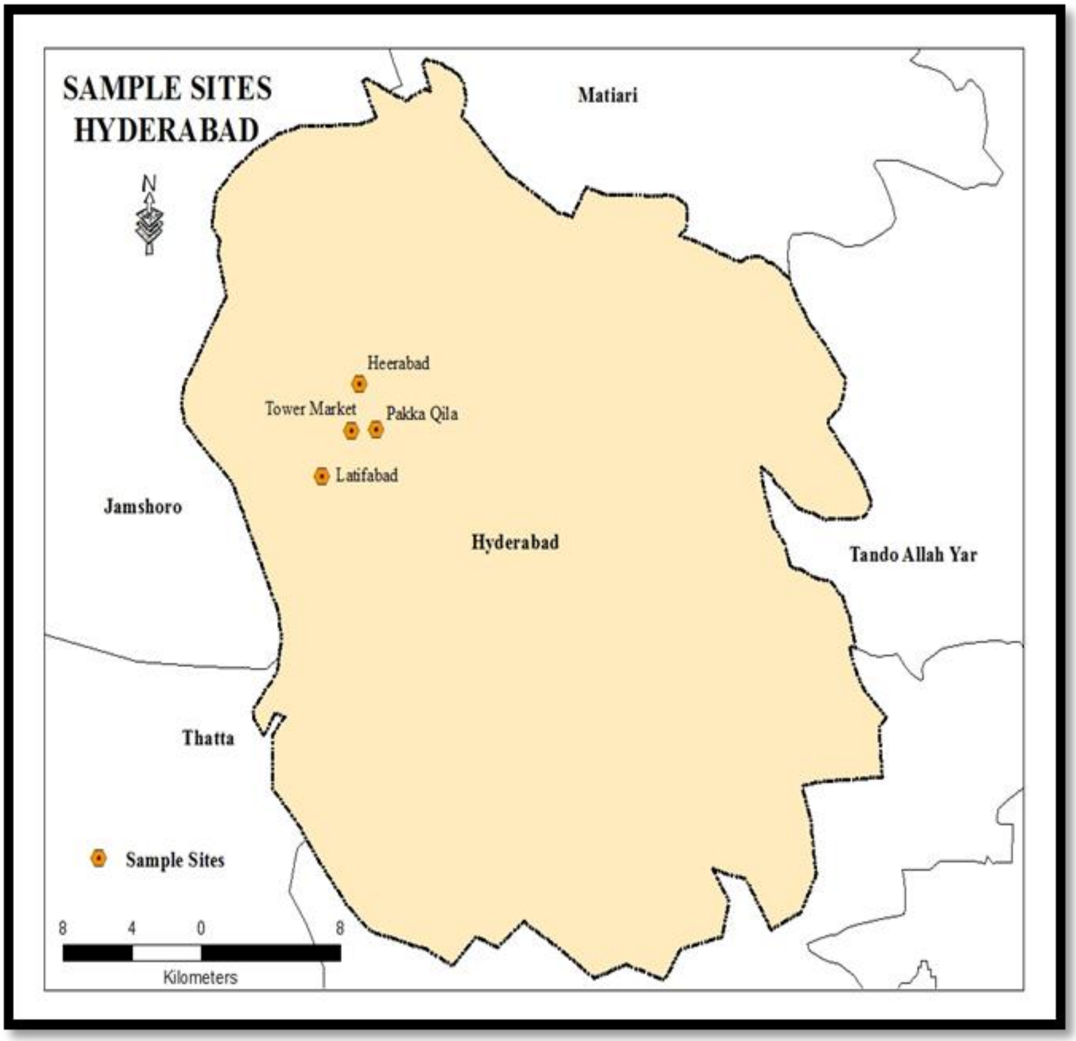

Fig. 2. Map of study areas of Hyderabad.

collected from selected areas of Karachi, Hyderabad and Thatta cities of Pakistan.

MATERIALS AND METHODS

Sampling Sites

Based on baseline study, 10 locations were selected in three major cities of Karachi, Hyderabad and Thatta (Figs**.** 1-3). Samples were collected from April 2011 to April 2014.

Sample Collection

A total of 135 samples, collected at random, of liver, meat, and blood of poultry chicken of 8 to 10 weeks of age having weight ranging from 1.2 to 1.5 kg. The liver and meat samples were collected in polyethylene bags and blood samples in anticoagulant violet top bottles. All samples were preserved at low temperature for determination of heavy metals.

Sample Preparation

The samples were analyzed using Atomic Absorption Spectroscopy. The instrument used was AAnalyst 700 from Perkin Elmer, USA.

Meat and Liver

Meat and liver samples were prepared according to the method of Khan *et al*. (2012). First the samples of meat and liver were homogenized separately. 10 grams of the homogenized sample was mixed with 6 ml of HNO_3. The mixture was kept in fume hood hotplate at 80°C for 6 hours until the mixture turned black. Then it was filtered using a whatman paper, 50 ml of deionized water was added and then mixture filtered again.

Whole Blood

Samples were prepared according to the method used by Nergis *et al*. (2000). First a solution of HNO_3 and HCl was prepared in the ratio of 20:1. Afterwards 0.5 ml of

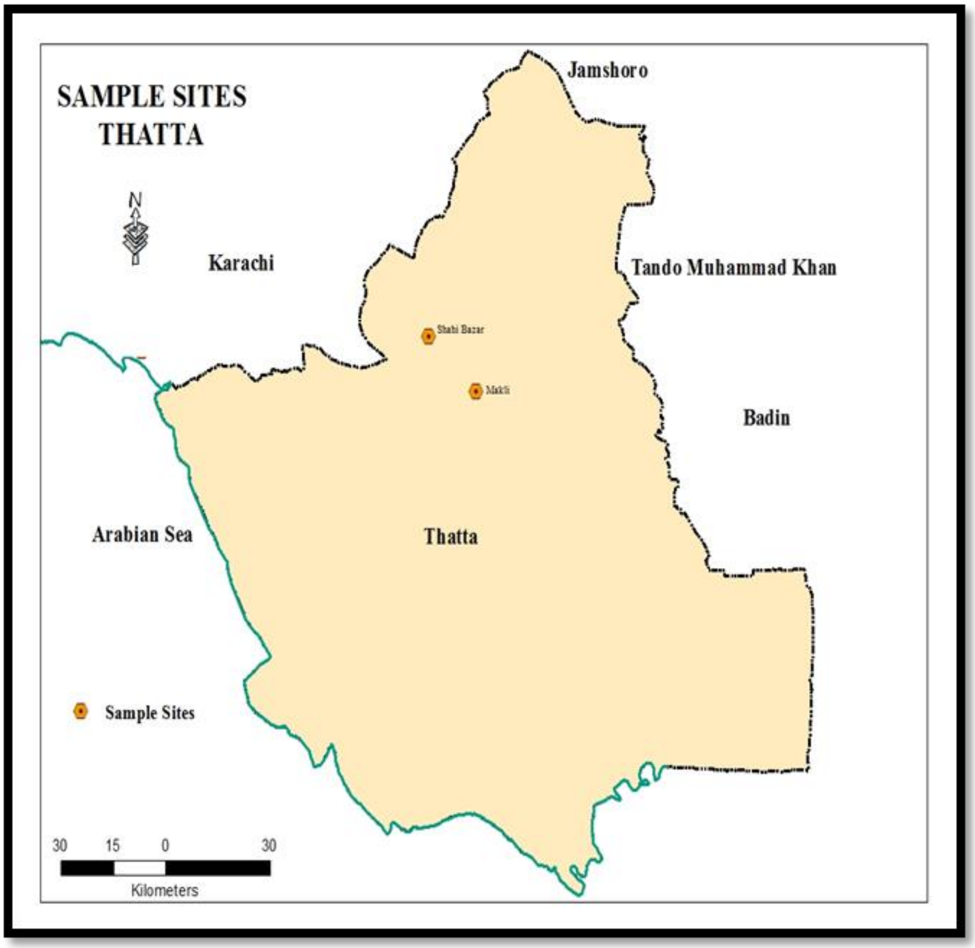

Fig. 3. Map of study areas of Thatta.

this solution was added to 1.5 ml of blood sample. To this, 10ml of distilled water was added and vortexed thoroughly. It was then heated at 60°C in water bath. When the sample reached half the original volume, 0.5 to 1 ml of HNO_3 was added to it. When the sample was cleared, it was filtered using 0.2mm filter and with distilled water the volume was made up to 25ml.

Preparation of Standards

1000ppm standards from Fisher UK were used. Standards of concentration 2, 4 and 6ppm were made by serial dilution using 1% Nitric acid. The equipment was calibrated with these standards and then the samples were analyzed. The analysis was done by aspirating into Atomic Absorption Spectrometer (Perkin Elmer AAnalyst 700) for detection of heavy metals Cadmium, Lead, Nickel, Zinc and Copper.

RESULTS AND DISCUSSION

Poultry meat is a food material being used largely in Pakistan. It is exposed to different heavy metals through poultry feed and environmental pollution in poultry breeding areas (Sattar and Chaudhary, 1978; Khurshid and Qureshi, 1984). The contamination of the food chain by heavy metals is getting important in view of its role in nutrition and human health. These heavy metals alter a number of parameters of the host's immune system and lead to increased susceptibility to infectious diseases. They are also capable of altering DNA structure (Bruins *et al*., 2000; Blasliak *et al*., 1999). The present study investigated the levels of heavy metals pollution in poultry chicken (*Gallus domesticus*) at Karachi, Hyderabad and Thatta cities of Sindh province.

Results in table 1 show the concentrations of heavy metals in liver samples of Karachi city Saddar (Downtown), Orangi Town, Lee Market and Clifton areas. The mean concentrations of Cadmium (Cd), Lead (Pb), Nickel (Ni), Zinc (Zn) and Copper (Cu) were found to be 0.103, 0.317, 6.169, 8.393 and 1.35ppm, respectively.

Table 1. Trace Metals concentration (ppm) mg/kg in Liver of Chicken *Gallus domesticus* in Karachi.

Sample No.	METALS				
	*Cd 228.8	*Pb 283.3	*Ni 232.0	*Zn 213.9	*Cu 324.8
1	0.205	0.317	5.945	9.035	1.670
2	0.205	0.497	6.780	8.395	1.835
3	0.105	0.920	6.305	9.435	1.540
4	0.095	0.575	6.368	7.036	2.155
5	0.085	0.155	7.546	9.865	1.925
6	0.016	0.720	5.445	8.025	N.D
7	0.250	0.550	6.252	8.390	N.D
8	0.009	0.220	6.303	9.457	0.587
9	0.225	0.140	6.250	7.802	1.905
10	0.075	0.119	5.840	7.225	2.202
11	0.035	0.182	5.900	7.440	1.825
12	0.045	N.D	8.203	9.203	0.440
13	0.130	0.120	4.302	8.325	
14	0.060	0.110	5.880	8.220	1.192
15	0.008	0.130	5.220	8.052	1.203

*Wavelength used for the detection of metals.

Table 2. Trace Metals concentration (ppm) mg/kg in Liver of Chicken *Gallus domesticus* in Hyderabad.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	N.D	1.105	6.523	5.267	1.765
2	N.D	1.685	5.063	7.995	1.237
3	N.D	0.952	7.445	6.045	1.955
4	0.025	1.325	6.187	4.257	3.048
5	0.008	1.110	6.408	N.D	1.192
6	0.031	0.853	6.953	6.920	1.225
7	0.061	0.255	7.025	7.095	2.207
8	0.015	0.220	7.327	6.880	2.956
9	0.003	1.105	6.203	4.958	3.015
10	0.010	1.320	6.752	4.809	1.660
11	N.D	1.207	5.095	7.065	1.138
12	N.D	0.854	5.345	7.030	2.056
13	0.035	0.352	6.201	6.550	2.010
14	0.018	1.225	7.225	7.660	1.228
15	0.011	1.275	7.510	7.545	1.220

Table 2, shows the concentrations of heavy metals in liver samples of Hyderabad city of Pakka Qilla, Tower Market, Heerabad and Latifabad areas. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.015, 0.99, 6.484, 6.005 and 1.861ppm, respectively.

The concentrations of heavy metals in the liver samples of Thatta city selected areas of Shahi Bazar and Makli showed in table 3. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.123, 1.234, 7.583, 6.044 and 2.254 ppm, respectively.

Table 4**,** shows the concentrations of heavy metals in meat samples of Karachi city Saddar, Orangi Town, Lee Market and Clifton areas. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.366, 1.797, 4.133, 6.414 and 0.363ppm, respectively. Table 5 shows the concentrations of heavy metals in meat samples of Hyderabad city Pakka Qilla, Tower Market, Heerabad and Latifabad areas. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.059, 0.991, 3.343, 4.539 and 0.357ppm, respectively.

Table 3. Trace Metals concentration (ppm) mg/kg in Liver of Chicken *Gallus domesticus* in Thatta.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	0.225	1.181	8.155	8.440	2.042
2	0.230	1.005	7.092	N.D	3.015
3	0.169	1.472	6.891	N.D	1.832
4	0.055	1.198	7.325	7.850	2.035
5	N.D	1.215	8.245	8.952	2.345
6	0.011	1.252	8.155	8.449	2.052
7	0.142	1.002	7.093	N.D	2.255
8	0.016	1.008	5.892	N.D	3.025
9	0.201	1.202	7,225	7.252	3.093
10	0.303	1.125	8.245	8.803	2.025
11	0.040	1.220	7.330	8.757	2.340
12	0.132	1.503	8.220	7.902	1.303
13	0.150	1.352	8.110	8.103	2.010
14	0.140	1.554	8.299	8.452	2.225
15	0.030	1.223	7.770	7.705	2.210

Table 4. Trace Metals concentration (ppm) mg/kg in Meat of Chicken *Gallus domesticus* in Karachi.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	0.245	1.825	2.592	7.165	0.362
2	0.695	2.273	3.035	6.732	0.365
3	0.482	2.652	4.484	7.225	0.373
4	0.365	4.565	3.575	5.852	0.345
5	0.305	5.715	4.355	6.585	0.375
6	0.250	1.009	5.408	6.175	N.D
7	0.690	0.222	4.255	6.252	N.D
8	0.502	N.D	3.701	7.230	N.D
9	N.D	0.020	4.882	5.903	0.275
10	0.007	N.D	5.205	6.595	0.340
11	0.143	1.725	3.095	5.229	0.225
12	0.503	1.850	4.506	6.480	0.280
13	0.325	0.186	3.275	5.225	0.360
14	0.250	0.097	5.280	7.340	0.306
15	N.D	1.225	4.350	6.225	0.752

Table 5. Trace Metals concentration (ppm) mg/kg in Meat of Chicken *Gallus domesticus* in Hyderabad.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	N.D	N.D	N.D	N.D	N.D
2	0.072	1.475	4.675	5.725	0.275
3	0.019	1.535	3.245	4.246	0.185
4	0.005	1.422	4.095	5.015	0.092
5	0.235	1.585	2.345	3.095	0.928
6	N.D	0.762	3.736	4.407	0.701
7	0.038	N.D	2.870	2.248	0.944
8	0.036	N.D	3.812	4.252	N.D
9	0.019	N.D	0.356	6.553	0.343
10	0.004	0.020	4.550	5.730	0.280
11	0.192	0.096	4.308	4.309	0.190
12	N.D	0.513	4.090	5.040	0.090
13	0.014	0.420	2.325	3.080	0.220
14	0.038	1.207	3.840	2.957	0.180
15	0.019	1.605	2.350	6.208	N.D

Table 6. Trace Metals concentration (ppm) mg/kg in Meat of Chicken *Gallus domesticus* in Thatta.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	0.282	1.690	4.205	5.339	0.297
2	0.445	1.565	3.576	4.065	0.245
3	0.245	1.593	4.997	4.685	0.232
4	0.136	1.465	3.485	3.635	0.251
5	0.232	1.582	3.345	4.095	0.927
6	0.143	0.513	4.205	4.035	0.005
7	0.448	1.030	3.575	4.065	0.958
8	0.450	1.026	5.061	4.785	0.252
9	0.253	1.607	6.025	3.775	0.751
10	0.007	1.565	5.275	3.085	0.321
11	N.D	1.752	6.225	5.258	0.302
12	N.D	1.950	4.210	4.756	0.204
13	0.041	1.565	3.752	3.955	0.105
14	0.005	1.551	4.757	3.085	0.905
15	0.101	1.602	3.245	5.095	0.956

Table 7. Trace Metals concentration (ppm) mg/l in Blood of Chicken *Gallus domesticus* in Karachi.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	N.D	0.166	0.306	6.116	0.866
2	N.D	0.0166	0.216	2.833	0.466
3	N.D	0.425	0.183	3.158	0.516
4	N.D	0.160	0.266	3.657	0.633
5	N.D	N.D	0.133	2.533	0.583
6	N.D	0.315	0.543	N.D	0.310
7	0.005	0.072	0.233	2.024	0.334
8	0.011	0.283	0.371	2.139	0.207
9	N.D	0.180	0.308	6.125	0.775
10	N.D	0.018	0.220	2.825	0.465
11	N.D	0.450	0.183	3.802	0.520
12	N.D	0.190	0.250	3.903	0.630
13	N.D	N.D	0.120	2.433	0.575
14	0.008	0.752	0.540	N.D	0.350
15	0.011	0.075	0.280	2.140	0.215

Table 6 shows the concentrations of heavy metals in meat samples of Thatta city Shahi Bazar and Makli areas. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.215, 1.47, 4.396, 4.248 and 0.447ppm, respectively. Table 7, shows the concentrations of heavy metals in blood of poultry chicken samples of Karachi city Saddar, Orangi Town, Lee Market and Clifton areas. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.009, 0.239, 0.277, 3.361 and 0.496ppm, respectively.

Table 8 shows the concentrations of heavy metals in blood samples of Hyderabad city Pakka Qilla, Tower Market, Heerabad and Latifabad areas. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.157, 0.045, 0.472, 4.078 and 0.502ppm, respectively, while table 9 shows the concentrations of heavy metals in blood samples of Thatta city Shahi Bazar and Makli areas. The mean concentrations of Cadmium, Lead, Nickel, Zinc and Copper were found to be 0.017, 0.298, 0.206, 3.028 and 0.368ppm, respectively. The maximum and minimum concentrations (ppm) of metals in different organs of all three locations are summarized in table 10.

Based on present study results, in Karachi city, Zinc showed the highest concentration in all three organs with 8.393 in liver, 6.6414 in meat and 3.361ppm in blood. According to the WHO criteria (FAO/WHO, 2000), the permissible concentration of Zinc level is 10-50ppm (mg/kg). In Hyderabad city, Ni showed the highest concentration in liver samples with mean concentration of 6.484ppm, while in meat and blood the highest concentration was of Zn, being 4.539 and 4.078 ppm, respectively. According to the WHO (1996) criteria the highest permissible limit of Ni is 0.5ppm (mg/kg).

Table 8. Trace Metals concentration (ppm) mg/l in Blood of Chicken *Gallus domesticus* in Hyderabad.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	0.012	N.D	0.861	4.523	0.489
2	0.014	N.D	0.098	5.208	0.505
3	0.031	N.D	0.310	6.281	0.545
4	0.932	N.D	0.152	7.301	N.D
5	0.125	N.D	0.195	2.887	0.119
6	0.041	0.054	0.736	4.432	0.701
7	0.038	N.D	0.870	2.240	0.945
8	0.036	N.D	0.812	4.280	N.D
9	0.019	N.D	0.356	6.578	0.343
10	0.025	N.D	0.870	3.543	0.475
11	0.008	N.D	0.075	3.428	0.510
12	0.095	0.055	0.325	2.892	0.541
13	0.753	0.045	0.175	3.753	N.D
14	0.180	0.025	0.885	2.892	N.D
15	0.039	N.D	0.360	3.427	0.345

Table 9. Trace Metals concentration (ppm) mg/l in Blood of Chicken *Gallus domesticus* in Thatta.

Sample No.	Cd	Pb	Ni	Zn	Cu
1	N.D	N.D	0.177	2.203	0.349
2	0.004	0.321	0.252	N.D	0.370
3	0.003	0.362	0.228	2.038	0.286
4	N.D	N.D	0.156	1.780	0.185
5	N.D	0.221	0.128	2.381	N.D
6	N.D	0.176	0.238	3.342	0.357
7	0.013	0.316	0.050	5.563	0.592
8	0.013	N.D	0.356	4.502	0.489
9	0.025	N.D	0.207	2.252	0.350
10	0.004	0.325	0.309	N.D	0.370
11	0.003	0.402	0.218	2.203	0.285
12	N.D	0.364	0.057	1.750	0.295
13	0.075	N.D	0.123	2.402	N.D
14	N.D	0.176	0.238	3.348	0.360
15	0.009	0.320	0.356	5.603	0.490

In Thatta city, Ni showed the highest concentration in both liver and meat, the concentrations being 7.538 and 4.396ppm, respectively, whereas in blood, Zn showed the highest concentrations of 3.028ppm. Among the three organs, liver contained the highest concentration of Ni, Zn and Cu, whereas Cd and Pb were found in the highest concentration in meat of chicken, while blood contained the lowest concentration of all metals except Cu, which was in lowest concentration found in meat.

Cadmium was found in the highest concentration i.e. 0.366ppm in meat of chicken collected from Thatta city, while its lowest concentration of 0.009ppm was found in the blood of chicken from Karachi city. The permissible limit for Cd in chicken is 0.5ppm (FAO/WHO, 2000). Cadmium has toxic effects and has been known to produce lung damage, kidney dysfunction, hepatic injury and hypertension (John and Jeanne, 1994). A recent study Sharmeen *et al.* (2014) reported the Cd concentrations in fish, *Oreochromis mossambicus,* was observed from 0.100 to 0.230ppm in the liver and 0.040 to 0.070ppm in the muscle. In the present study, the average concentrations of Cd in liver samples of chicken were found to be 0.103 in Karachi city, 0.015 in Hyderabad city and 0.123ppm in Thatta city, whereas in meat samples were 0.366ppm in Karachi city, 0.046 in Hyderabad city and 0.215ppm in Thatta city. Lead contamination has been observed in the meat samples of chicken in Karachi city, showed the highest level i.e. 1.797ppm, while the lowest concentration of 0.045ppm was observed in the blood of chicken from Hyderabad city which is very high as compared to the WHO (1996) permissible limit of 0.2ppm, these results show serious contamination of chicken meat and blood. Humans are exposed to lead mainly through gasoline, contaminated water, food and lead in paint (NAPE, 1993).

Table 10. Summary of maximum and minimum concentrations (ppm) of metals in different organs of all three locations.

Cities	METALS						
	Organs		Cd	Pb	Ni	Zn	Cu
			**0.5 (FAO, WHO)	**0.2 (WHO)	**0.5 (WHO)	**10-50 (FAO, WHO)	**200 (ANZFA)
KARACHI							
	Liver	Maximum	0.250	0.920*	8.203*	9.865	2.202
		Minimum	0.008	0.110	4.302	7.036	0.440
	Meat	Maximum	0.695*	5.715*	5.408*	7.340	0.752
		Minimum	0.007	0.020	2.592	5.225	0.225
	Blood	Maximum	0.011	0.752*	0.543*	6.125	0.866
		Minimum	0.005	0.016	0.120	2.024	0.207
HYDERABAD							
	Liver	Maximum	0.061	1.685*	7.510*	7.995	3.048
		Minimum	0.003	0.220	5.063	4.257	1.138
	Meat	Maximum	0.072	1.605*	4.675*	6.553	0.928
		Minimum	0.004	0.020	0.356	2.248	0.090
	Blood	Maximum	0.932*	0.055	0.885*	7.301	0.945
		Minimum	0.012	0.025	0.075	2.240	0.119
THATTA							
	Liver	Maximum	0.303	1.554*	8.299*	8.952	3.093
		Minimum	0.011	1.002	5.892	7.252	1.303
	Meat	Maximum	0.450	1.950*	6.225*	5.339	0.958
		Minimum	0.005	0.513	3.245	3.085	0.005
	Blood	Maximum	0.075	0.402*	0.356	5.603	0.592
		Minimum	0.003	0.176	0.050	1.750	0.185

*Concentrations which exceed the maximum permissible limit prescribed by WHO, FAO and ANZFA. ** Permissible Limit

The chicken samples of Thatta showed highest concentration of Nickel, in the liver 8.299, 3.245ppm in meat, while the lowest concentration was observed in the blood 0.356ppm. The present study results showed that the concentration of Nickel in the liver and meat of chicken is higher compared to the permissible limit of 0.5 ppm (WHO, 1996), and is indicative of Nickel contamination in relatively high proportion. Nickel is discharged from many sources like petroleum refineries, electroplating units, steel, fertilizers, automobiles and batteries and nickel mining (Duke *et al.,* 1990). Nickel can cause respiratory problems and is a carcinogen as well (Agency for Toxic Substance and Disease Registry, 2004).

In the present study, Zinc concentration was found to be highest in the liver from Karachi city chicken samples i.e. 8.393ppm and lowest in the blood of Thatta chicken samples i.e. 3.028ppm. The permissible limit for Zinc in chicken is 10-50ppm (FAO/WHO, 2000). Zinc mainly causes phototoxicity in plants and relatively low toxicity in animals and human. Industrial wastes are responsible for an increase of this metal in water (Alloway and Ayres, 1994).

Copper was found in highest concentration in liver samples of chicken Thatta city i.e. 2.254 and in lowest in meat of chicken from Hyderabad city i.e. 0.357ppm. The permissible limit for Copper in chicken is 200ppm (ANZFA, 2001). The source of metals in the environment is the combustion of fossil fuels, mining industries, waste disposal and domestic sewage. Farming and forestry also contribute to the metal content in the environment via fertilizers and pesticides (Friberg *et al*., 1979). Another study, Sharmeen *et al.* (2014) reported that concentrations of Copper in fish, *Oreochromis mossambicus,* ranged from 2.050 to 5.080ppm in liver and 0.190 to 0.400ppm in the muscle.

An earlier study by Khan *et al*. (2012) reported that all chickens samples collected from Karachi, Hyderabad and Thatta city were contaminated with Cadmium, Nickel, Copper and Lead. Whereas Copper contamination was found to be alarming, touching the highest level as compared to other metals tested. In the present study, average concentrations of Copper in liver of chicken samples were found to be 1.35ppm in Karachi, 1.861ppm in Hyderabad and 2.254ppm in Thatta city, whereas in meat were 0.363ppm in Karachi and 0.357ppm in Hyderabad and 0.447ppm in Thatta city samples.

CONCLUSION

Exposure of toxic contaminants is one of the major risk factors leading to many diseases. The present study concluded that toxicity being developed with heavy metals (Cadmium, Lead, Nickel, Zinc and Copper) contamination is quite hazardous for human health. The contamination level of Nickel and Lead exceeded the maximum permissible limit set by WHO and ANZFA. This can be extremely detrimental and critical, keeping in view the toxic nature and extreme negative effects of these heavy metals on the humans, and the high use of poultry meat as a food source in our country. The contaminated poultry feed is transferred to chicken through ingestion (Khan *et al*., 2012) well substantiated by our study and analysis of several feed samples. It is recommended that immediate and effective measures be taken by the Government and the health quality control authorities so as to ensure the hygiene of the poultry feed processing areas and also to minimize environmental sources of heavy metal contamination.

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