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DETERMINATION OF HEAVY METALS IN BRAIN, LIVER AND HEART MUSCLES OF POULTRY CHICKEN *GALLUS DOMESTICUS* IN THREE CITIES OF SINDH

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ABSTRACT

In the present study, the level of heavy metals pollution in poultry chicken *Gallus domesticus* at Karachi, Hyderabad and Thatta were investigated during 2007-2009. A total of 200 random poultry organs (liver, brain and heart) of the same aged groups (12 weeks old) of chickens were collected from poultry shops in 6 selected locations, and four metals Cd, Ni, Cu and Pb were analyzed. Based on the results all chicken samples were contaminated with Cadmium (Cd), Nickel (Ni), Copper (Cu) and Lead (Pb), whereas Copper had the highest contamination level of all four metals tested. Based on poultry feeds sample analysis, metals may have likely been transferred to Chicken via ingestion of these contaminated poultry feed. Observations made at Karachi poultry feed processing factories showed unhygienic and poor environment which needs improving and revised regulations of the feed processing centers.

Keywords: Poultry chicken, heavy metals, cadmium, copper, nickel, lead.

INTRODUCTION

Our earth's crust is the ultimate source of all metallic elements present in the ecosystem. Globally there are 35 metals that concern us because of residential or occupational exposure; 23 of these are the heavy metals: Cadmium, Copper, Lead, Nickel, Zinc, Antimony, Arsenic, Bismuth, Cerium, Chromium, Cobalt, Gallium, Gold, Iron, Manganese, Mercury, Platinum, Silver, Tellurium, Thallium, Tin, Uranium, and Vanadium (Glanze, 1996).

Some trace elements like copper, nickel, zinc, cobalt, manganese, and selenium are structurally part of important molecules and act as cofactors of enzymes in the metabolic process, while excessive concentrations of these elements are toxic (Chang and Cockerham, 1994).

Metal contamination in food additives or land food was keenly noticed in previous decades. Thus the issue of contamination in food and water became of great concern to health authorities. As a result WHO have proposed some optimum levels of metals in food and drinking water.

According to the WHO experts, there should be a complete analysis of diet pattern, so that, every product could be analyzed individually, for its contribution of metal toxicity is total metal contamination. The major factors that are involved in accumulation of heavy metals in edible products are natural conditions, fertilizers and industrial pollution. Since climatic conditions, soil texture and other related environmental factors differ greatly according to the area, the concentration of heavy metals also fluctuates from place to place and region to region. The estimation of population exposure to these contaminants can easily be evaluated from marketed products.

It is already known that beyond a certain level all metals are poisonous for human beings. Industrial effluents are mainly carcinogenic or mutagenic in nature due to the presence of high concentrations of heavy metals and hazardous chemicals (Gilberg, 1974; Degraeve, 1981, Moore and Ramamoorthy, 1984; Chong *et al.*, 1998; Hamilton *et al*., 1998; Zhitkovich *et al*., 1998; Roberts, 1999; Underwood, 1979). The presence of heavy metals within body's tolerance is non-toxic and combination with body's protein is a natural process; but these heavy metals can cause pathogenicity if they exceed the tolerance level (Jaffer, 1988). Toxicity of both essential and non-essential metals can occur if their concentrations are increased in the body. This can result in destruction of cell membranes and alteration of enzymatic and molecular functions. These metals are also capable of altering the DNA structure (Bruins *et al*., 2000; Blasliak *et al*., 1999).

Mostly the agriculture sector and natural vegetation suffer from the devastating effects of industrial and domestic pollution. Pollution is also considered to be a serious

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health problem and can sometimes be lethal. Normally heavy metals become toxic when they accumulate in the soft tissues of the body. The main contributors of cadmium and zinc pollution are commercial fertilizers such as phosphate fertilizers. These fertilizers are an importance source of cadmium in different vegetable species. While super-phosphate fertilizers increase the concentration of cadmium in Seoul, cereals and fodder plants (Schroefer and Blassa, 1963).

Cadmium (Cd) is a silver-white, ductile, very malleable metal, and levels in the environment vary widely and are an important part of the environment. Normally human uptake of cadmium takes place through food. Foodstuffs that are rich in cadmium can greatly increase the cadmium concentration in human bodies. Plants cultivated from industrial sites, may only exhibit tolerable levels of cadmium, but on the other hand, animals are found greatly affected, especially their visceral organs, such as, liver and kidneys. Cigarettes are another source of Cadmium contamination, as lungs absorb cadmium residues more effectively, than the stomach absorbs them from food.

Nickel (Ni) is silvery-white hard, malleable, and ductile metal, and a 24th element in order of natural abundance in the earth's crust, and it is widely distributed in the environment (Kasprzak *et al*., 2003). Nickel is used in many industrial and consumer products, including stainless steel, magnets, coinage and special alloys. It is also utilized for plating and as a green tint in glass. Nickel is a naturally occurring metal existing in found oceans, rivers, wetlands, air, soil, drinking water, animals and plants. Naturally foodstuffs contain small amounts of nickel, while chocolate and fats are known to contain severely high quantities. Nickel is an important from a biochemical point of view, due to the decreased stability of its higher oxidation states (Atkins, 1978). Nickel is considered to be an essential element, required for proper functioning of the liver in rats and chicks, and also in the maintenance of their morphology, but its continuous exposure may cause respiratory cancer. Nickel contamination produces lethal effects on humans, characterized mainly of pulmonary disorders. Its toxicity causes lipid per oxidation in target organs (Nielson, 1991).

Lead (Pb) is a bluish-white lustrous metal and one of the elements in nature. The main sources of lead exposure include lead in paint, gasoline, water, and food (NAPE, 1993). Lead is a health hazard for all humans (Carolyn, 2010; ATSDR, 1988). Many vegetables, fruits, meats, grains, seafood, and soft drinks may contain some amounts of lead.

Copper (Cu) is a reddish metal with a face-centered cubic crystalline structure, and an essential substance to human life because copper is a trace element that is essential for human health, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from copper pipes, as well as from additives designed to control algal growth. Copper does not break down in the environment and because of that it can accumulate in plants and animals when it is found in soils. Copper can be released into the environment by human activities and natural sources. The higher concentration of Copper in water system, greatly affects the marine ecosystem. It usually alters the functions of gills, liver, kidneys and nervous system, specifically, the sense of smell in fishes, due to which hindrance is produced in their ability of choosing an appropriate mate or in the detection of mating sites. It has been found that Cu toxicity may cause chromosomal mutation which leads to inborn disorders in fish (Sarkar *et al*., 1983).

Heavy metal contamination of the environment arises not only naturally, but from industrial activities. Currently Pakistan is facing some environmental pollution problems including heavy metals pollution. Karachi has two industrial estates, but there is no effluent treatment plant, and industrial products and used material may contain high concentrations of toxic metals. In small quantities, many heavy metals such as manganese, copper, iron, and zinc are nutritionally important for a healthy life, these are referred to as the trace elements. The use of food and food additives may represent the biggest source of exposure to heavy metals (Shohda *et al.*, 2001).

The objective of the present study was to study some selected heavy metals (cadmium, lead, copper and nickel) level of the brain, liver and heart muscle of poultry chicken *Gallus domesticus* in Karachi, Hyderabad and Thatta cities by province of Sindh.

MATERIALS AND METHODS

Study areas

Three Chicken organs, liver, brain and heart were collected from poultry shops in three cities, Karachi, Thatta and Hyderabad during the period of June 2007 – May 2009.

Sampling Sites

After baseline study four locations: Station A (North Karachi, SITE, Nazimabad, Gabol town), Station B (Lasbella, Liaquatabad, Grumandir, Empress Market), Station C (Malir, Landhi, Korangi Industrial area), Station D (Garden, Lyari and Lee Market), Station E (Hyderabad city), and Station F (Thatta city) were selected (Figs. 1-3):

Method of Samples Collected

A total of 200 random poultry organs (liver, brain and heart) from the same aged groups (12-weeks) of chickens

were collected from poultry shops of selected locations. Thirty poultry feed samples were also collected from all locations and feed Mills in Karachi, Thatta and Hyderabad.

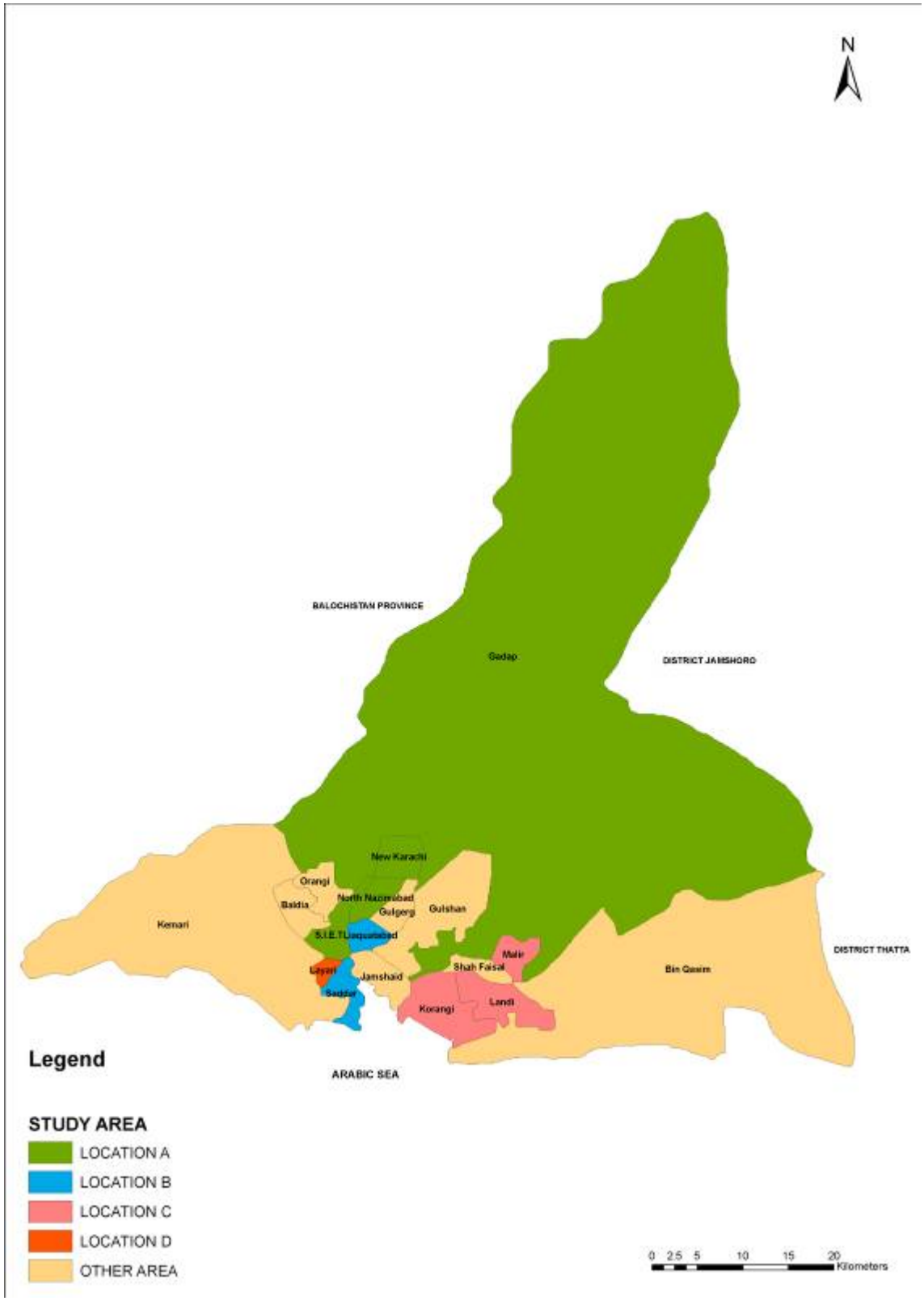

Fig. 1. Map of study areas of Karachi city.

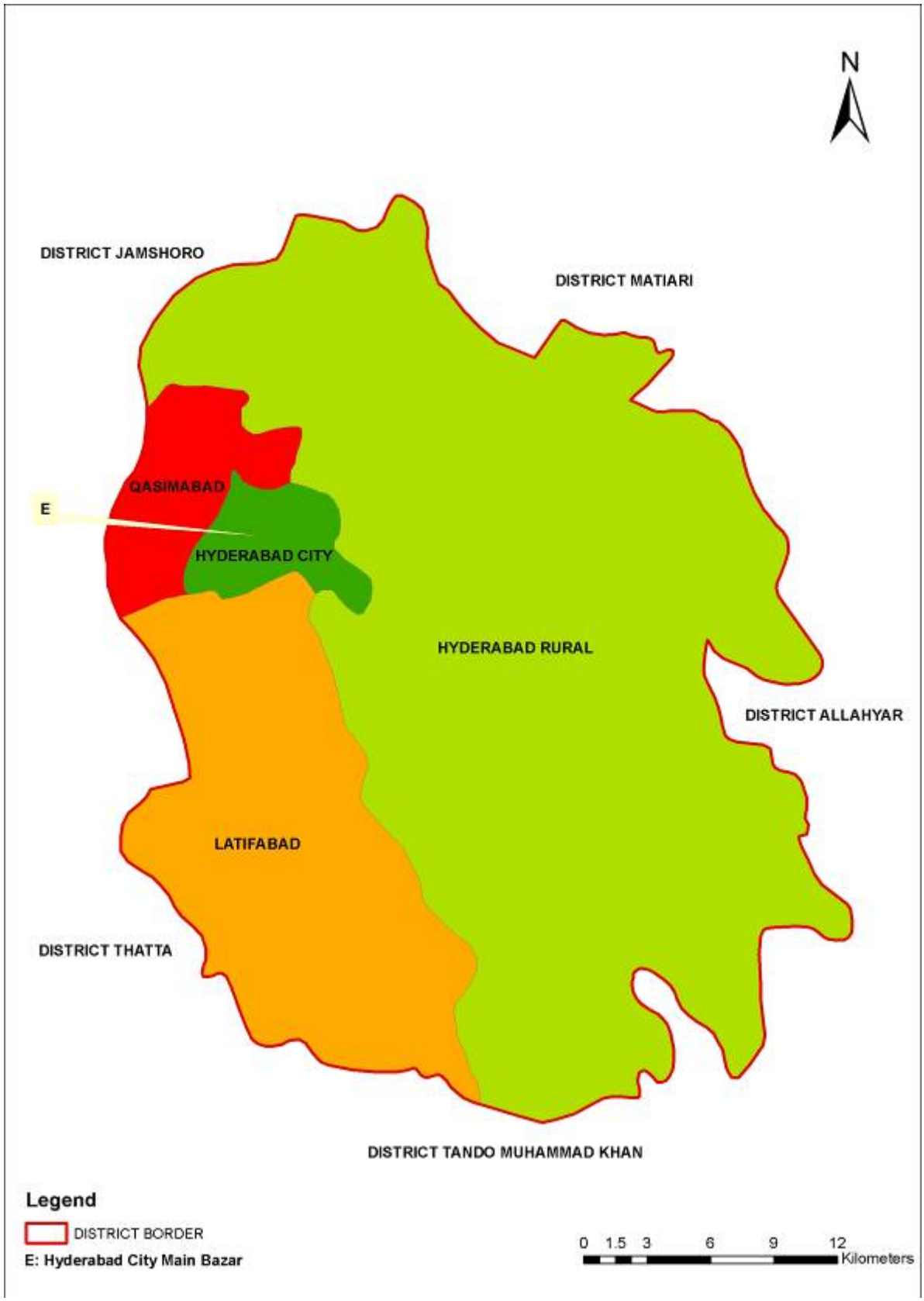

Fig. 2. Map of study areas of Hyderabad city.

Fig.3. Map of study areas of Thatta city.

Preservation of the Samples
Liver, brain, heart, and feeds samples were collected in polyethylene bags from various poultry shops. The poultry samples were stored at -10^{o}C for analysis of trace metals.

Preparation and Analysis of samples
Sample
Samples of liver, brain, heart and poultry feeds were homogenized separately. 5gm of the fresh homogenate were evaporated to dry in an oven at 100^{o}C according to Crosby (1977) methods.

Digestion
Dried 1gm of sample of each organ/ poultry feed were transferred to a 250ml glass beaker and acidified by adding 1:3 $HClO_4$ 65% and HNO_3 60% and digested at 90^{o}C in hot plate.

Preparation of standard solution
Following stock standard solutions (1000pm) were prepared by dissolving required amounts of the respective salt in deionizer water:
1). Cadmium chloride ($CdCl_2$),
2). Copper sulphate ($CuSO_4$),
3). Lead nitrate $Pb(NO_3)_2$
4). Nickel chloride ($Nicl_2$)

Dilutions of the stock solutions were made to prepare working standards in the measuring ranges. A blank was also prepared and analyzed. Blank consisted of the same amounts of all chemicals into unknown treat were used in an analysis in any (including water). This blank was run through the entire analytical procedure. The blank results were subtracted from the analytical. Sample results for obtaining the net concentration in the sample solution.

In order to obtain high precision of the analysis, replicate samples were taken for analysis; this reliability of the result was ascertained.

The following heavy metals were determined in poultry organs and feed:
1) Cadmium Cd
2) Lead Pb
3) Copper Cu
4) Nickel Ni
An atomic absorption spectrophotometer ICAP 6500 D40 ICP was used for the analysis.

Recovery experiments
For the confirmation of results, recovery experiments were also conducted.

RESULTS AND DISCUSSION

Environmental pollution has become a worldwide concern and it affects the ecological systems and human health. The indiscriminate release of harmful chemicals especially toxic metals in an environment can adversely affect the quality of different food resources. Chicken is a healthy alternative to red meat and the primary source of animal protein.

According to results, during the period 2007-8, samples from station A (North Karachi, SITE, Nazimabad, and Gabol town), the mean concentrations of Cadmium (Cd), Nickel (Ni), Copper (Cu) and Lead (Pb) in liver were found between 0.104, 0.023, 0.767 and 0.060 ppm, respectively, while in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found 0.199, 0.022, 0.168 and 0.009ppm, respectively.

In the year 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in the brain were found in 0.105, 0.320, 0.050 and 0.040 ppm, respectively. While during the period 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.125, 0.297, 0.040 and 0.041 ppm, respectively. During the period 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in heart muscles were found 0.113, 0.092, 0.161 and 0.036 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.110, 0.067, 0.147 and 0.040 ppm, respectively. Table 1 shows the maximum and minimum concentrations of four selected metals.

During the period 2007-8, samples of station B (Lasbella, Liaquatabad, Grumandir and Empress Market), the mean concentrations of Cd, Ni, Cu and Pb in liver were found to be 0.198, 0.007, 0.142 and 0.010 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.141, 0.001, 0.171 and 0.008 ppm, respectively. In 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in brain muscles were found in 0.002, 0.002, 0.060 and 0.046 ppm, respectively. While during the period 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found 0.003, 0.002, 0.057 and 0.040 ppm, respectively. During the period 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in heart muscles were found in 0.009, 0.015, 0.539 and 0.049 ppm, respectively. While in year 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.009, 0.009, 0.526 and 0.055 ppm, respectively. The maximum and minimum concentration levels of metals were summarized in table 2.

During the period 2007-8, samples of station C (Malir, Landhi, Korangi Industrial area), the mean concentrations of Cd, Ni, Cu and Pb in liver were found in 1.020, 1.012, 2.522 and 1.054 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 1.016, 1.019, 2.486 and 1.061 ppm, respectively. In the year 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in brain muscles were found in 0.003, 0.001, 0.061 and 0.051 ppm, respectively. While in 2008-9, the mean

Table 1. Summary of maximum and minimum metal concentration levels (ppm) at station A, 2007-2009.

Organs	Years	Concentration (ppm)	Metals Cd	Ni	Cu	Pb
Liver	June-2007 to May-2008					
		Maximum	0.112	0.027	0.771	0.068
		Minimum	0.098	0.021	0.762	0.052
Liver	June-2008 to May-2009					
		Maximum	0.204	0.022	0.175	0.032
		Minimum	0.194	0.021	0.162	0.003
Brain	June-2007 to May-2008					
		Maximum	0.114	0.468	0.068	0.042
		Minimum	0.001	0.300	0.042	0.038
Brain	June-2008 to May-2009					
		Maximum	0.132	0.302	0.042	0.045
		Minimum	0.114	0.294	0.038	0.037
Heart	June-2007 to May-2008					
		Maximum	0.126	0.097	0.170	0.041
		Minimum	0.109	0.087	0.157	0.031
Heart	June-2008 to May-2009					
		Maximum	0.114	0.076	0.148	0.042
		Minimum	0.106	0.061	0.145	0.037

Table 2. Summary of maximum and minimum metal concentration levels (ppm) at station B, 2007-2009.

Organs	Years	Concentration (ppm)	Metals Cd	Ni	Cu	Pb
Liver	June-2007 to May-2008					
		Maximum	0.201	0.008	0.145	0.011
		Minimum	0.177	0.005	0.138	0.008
Liver	June-2008 to May-2009					
		Maximum	0.145	0.003	0.176	0.009
		Minimum	0.139	0.001	0.168	0.007
Brain	June-2007 to May-2008					
		Maximum	0.003	0.003	0.062	0.049
		Minimum	0.001	0.001	0.059	0.042
Brain	June-2008 to May-2009					
		Maximum	0.004	0.003	0.061	0.041
		Minimum	0.001	0.001	0.054	0.040
Heart	June-2007 to May-2008					
		Maximum	0.010	0.017	0.540	0.052
		Minimum	0.008	0.012	0.450	0.047
Heart	June-2008 to May-2009					
		Maximum	0.010	0.011	0.531	0.056
		Minimum	0.008	0.008	0.520	0.052

concentrations of Cd, Ni, Cu and Pb were found in 0.005, 0.001, 0.061 and 0.040 ppm, respectively. During the period 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in heart muscles were found in 0.172, 0.120, 0.846 and 0.161 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.177, 0.120, 0.961 and 0.163 ppm, respectively. The maximum and minimum concentration levels of four selected metals were summarized in table 3.

Table 3. Summary of maximum and minimum metal concentration levels (ppm) at station C, 2007-2009.

Organs	Years	Concentration (ppm)	Metals Cd	Ni	Cu	Pb
Liver	June-2007 to May-2008					
		Maximum	1.098	1.012	2.524	1.056
		Minimum	1.018	1.011	2.520	1.051
Liver	June-2008 to May-2009					
		Maximum	1.055	1.020	2.490	1.062
		Minimum	1.015	1.016	2.482	1.057
Brain	June-2007 to May-2008					
		Maximum	0.005	0.002	0.064	0.052
		Minimum	0.002	0.001	0.057	0.049
Brain	June-2008 to May-2009					
		Maximum	0.006	0.002	0.062	0.042
		Minimum	0.004	0.001	0.059	0.038
Heart	June-2007 to May-2008					
		Maximum	0.175	0.121	0.848	0.165
		Minimum	0.168	0.118	0.842	0.155
Heart	June-2008 to May-2009					
		Maximum	0.180	0.124	0.968	0.164
		Minimum	0.175	0.119	0.953	0.162

Table 4. Summary of maximum and minimum metal concentration levels (ppm) at station D, 2007-2009.

Organs	Years	Concentration (ppm)	Metals Cd	Ni	Cu	Pb
Liver	June-2007 to May-2008					
		Maximum	0.189	0.027	0.536	0.061
		Minimum	0.178	0.017	0.518	0.058
Liver	June-2008 to May-2009					
		Maximum	0.178	0.034	0.600	0.069
		Minimum	0.160	0.021	0.583	0.050
Brain	June-2007 to May-2008					
		Maximum	0.002	0.002	0.069	0.037
		Minimum	0.001	0.001	0.038	0.031
Brain	June-2008 to May-2009					
		Maximum	0.006	0.001	0.065	0.040
		Minimum	0.004	0.001	0.048	0.034
Heart	June-2007 to May-2008					
		Maximum	0.078	0.023	0.702	0.069
		Minimum	0.074	0.018	0.612	0.065
Heart	June-2008 to May-2009					
		Maximum	0.069	0.021	0.621	0.088
		Minimum	0.066	0.019	0.591	0.065

During the period 2007-8, samples of station D (Garden, Lyari and Lee Market), the mean concentrations of Cd, Ni, Cu and Pb in liver were found in 0.180, 0.022, 0.529 and 0.059 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.172, 0.027, 0.594 and 0.055 ppm, respectively. In 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in brain muscles were found in 0.001, 0.001, 0.051 ppm and 0.034 respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.005, 0.001, 0.055 and 0.036 ppm, respectively. During the period 2007-8, the mean concentrations of Cd, Ni, Cu and Pb in heart

Table 5. Summary of maximum and minimum metal concentration levels (ppm) at station E, 2007-2009.

Organs	Years	Concentration (ppm)	Metals Cd	Ni	Cu	Pb
Liver	June-2007 to May-2008					
		Maximum	0.041	0.020	0.132	0.080
		Minimum	0.036	0.014	0.126	0.025
Liver	June-2008 to May-2009					
		Maximum	0.042	0.023	0.428	0.101
		Minimum	0.039	0.020	0.391	0.084
Brain	June-2007 to May-2008					
		Maximum	0.001	0.001	0.052	0.034
		Minimum	0.001	0.001	0.049	0.030
Brain	June-2008 to May-2009					
		Maximum	0.001	0.001	0.056	0.041
		Minimum	0.001	0.001	0.048	0.038
Heart	June-2007 to May-2008					
		Maximum	0.050	0.017	0.548	0.071
		Minimum	0.041	0.013	0.458	0.051
Heart	June-2008 to May-2009					
		Maximum	0.071	0.023	0.714	0.063
		Minimum	0.061	0.020	0.643	0.060

Table 6. Summary of maximum and minimum metal concentration levels (ppm) at station F, 2007-2009.

Organs	Years	Concentratin (ppm)	Metals Cd	Ni	Cu	Pb
Liver	June-2007 to May-2008					
		Maximum	0.222	0.024	0.546	0.074
		Minimum	0.208	0.023	0.538	0.068
Liver	June-2008 to May-2009					
		Maximum	0.215	0.032	0.542	0.069
		Minimum	0.204	0.023	0.534	0.050
Brain	June-2007 to May-2008					
		Maximum	0.002	0.002	0.038	0.042
		Minimum	0.001	0.001	0.030	0.039
Brain	June-2008 to May-2009					
		Maximum	0.002	0.005	0.042	0.042
		Minimum	0.001	0.001	0.038	0.035
Heart	June-2007 to May-2008					
		Maximum	0.069	0.024	0.069	0.089
		Minimum	0.065	0.018	0.039	0.079
Heart	June-2008 to May-2009					
		Maximum	0.070	0.024	0.053	0.070
		Minimum	0.068	0.021	0.049	0.064

muscles were found in 0.075, 0.021, 0.668 and 0.068 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.068, 0.020, 0.600 and 0.082 ppm, respectively. The maximum and minimum concentration levels of metals were summarized in table 4.

During the period 2007-8, samples of station E ((Hyderabad city), the mean concentrations of Cd, Ni, Cu and Pb were found in liver 0.039, 0.016, 0.128 and 0.078 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.040, 0.021, 0.400 and 0.096 ppm, respectively. In 2007-8, the

Table 7. Average metal concentrations (ppm) in Liver, Brain and Heart of *Gallus domesticus* at Stations A-F from June 2007 to May 2008.

	Metals				
	Organs	**Cd**	**Ni**	**Cu**	**Pb**
	Liver	0.104	0.023	0.767	0.060
Station A	Brain	0.105	0.320	0.050	0.040
	Heart	0.113	0.092	0.161	0.036
	Liver	0.198	0.007	0.142	0.010
Station B	Brain	0.002	0.002	0.060	0.046
	Heart	0.009	0.015	0.539	0.049
	Liver	1.020	1.012	2.522	1.054
Station C	Brain	0.003	0.001	0.061	0.051
	Heart	0.172	0.120	0.846	0.161
	Liver	0.180	0.022	0.529	0.059
Station D	Brain	0.001	0.001	0.051	0.034
	Heart	0.075	0.021	0.668	0.068
	Liver	0.039	0.016	0.128	0.078
Station E	Brain	0.001	0.001	0.051	0.031
	Heart	0.047	0.015	0.547	0.061
	Liver	0.211	0.024	0.541	0.070
Station F	Brain	0.001	0.001	0.035	0.041
	Heart	0.068	0.022	0.051	0.083

mean concentrations of Cd, Ni, Cu and Pb were found in brain muscles 0.001, 0.001, 0.051 and 0.031 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.001, 0.001, 0.050 and 0.039 ppm, respectively. During the 2007-8, the mean concentrations of Cd, Ni, Cu and Pb were found in heart muscles 0.047, 0.015, 0.547 and 0.061 ppm, respectively. While during the 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.069, 0.022, 0.684 and 0.062 ppm, respectively. The maximum and minimum concentrations of selected metals were summarized in table 5.

During the period 2007-8, samples of station F (Thatta city), the mean concentrations of Cd, Ni, Cu and Pb were found in liver 0.211, 0.024, 0.541 and 0.070 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.211, 0.028, 0.539 and 0.056 ppm, respectively. In 2007-8, the mean concentrations of Cd, Ni, Cu and Pb were found in brain muscles 0.001, 0.001, 0.035 and 0.041 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.001, 0.003, 0.041 and 0.039 ppm, respectively. During the period 2007-8, the mean concentrations of Cd, Ni, Cu and Pb were found in heart muscles 0.068, 0.022, 0.051 and 0.083 ppm, respectively. While in 2008-9, the mean concentrations of Cd, Ni, Cu and Pb were found in 0.069, 0.022, 0.051 and 0.068 ppm, respectively. The maximum and minimum concentrations of metals were summarized in table 6.

Heavy metals contamination is a serious problem because of their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Uruc, 2006). The risk associated with the exposure to heavy metals present in food products had aroused widespread concern in human health. Chicken is an important source of protein in our country and widely consumed in all four provinces. The major source of heavy metals in chicken arises from contamination of poultry feeds. In the present study concentrations of Cd, Ni, Cu and Pb were observed in the liver, brain and heart muscles of chicken (*Gallus domesticus*) of three cities by province of Sindh, biggest and populated city of Karachi, 3rd largest city Hyderabad and historical small city Thatta during 2007-2009.

Table 8. Average metal concentrations (ppm) in Liver, Brain and Heart of *Gallus domesticus* at Stations A-F from June 2008 to May 2009.

	Metals				
	Organs	**Cd**	**Ni**	**Cu**	**Pb**
	Liver	0.199	0.022	0.168	0.009
Station A	Brain	0.125	0.297	0.040	0.041
	Heart	0.110	0.067	0.147	0.040
	Liver	0.141	0.001	0.171	0.008
Station B	Brain	0.003	0.002	0.057	0.040
	Heart	0.009	0.009	0.526	0.055
	Liver	1.016	1.019	2.486	1.061
Station C	Brain	0.005	0.001	0.061	0.040
	Heart	0.177	0.120	0.961	0.163
	Liver	0.172	0.027	0.594	0.055
Station D	Brain	0.005	0.001	0.055	0.036
	Heart	0.068	0.020	0.600	0.082
	Liver	0.040	0.021	0.400	0.096
Station E	Brain	0.001	0.001	0.050	0.039
	Heart	0.069	0.022	0.684	0.062
	Liver	0.211	0.028	0.539	0.056
Station F	Brain	0.001	0.003	0.041	0.039
	Heart	0.069	0.022	0.051	0.068

At the station A, Copper had high level concentrations in the liver 0.771ppm during year 2007-8, while in year 2008-9 Cadmium showed higher amount 0.204 ppm in the same organ. Cadmium ranks close to lead and mercury as one of the top toxic substances (Klaassen, 2008) and permissible concentration limit of Cd is 0.5 mg/kg (Anonymous, 2000). In 2007, 2008 and 2009, Ni was showing 0.468 and 0.302 ppm high concentrations in the brain. In 2007-8 and 2008-9, both years Cu was found 0.170 and 0.148 ppm concentration in the heart as high level as compared to other three metals. The allowable value of Ni for human is 40mg/kg. Station A consists of North Karachi, SITE, Nazimabad, and Gabol town. SITE is an industrial area where environmental contamination is very high due to dumping of waste material without proper recycling process.

Station B, Cd showed high level concentrations in the liver 0.201 ppm during the year 2007-8, while in year 2008-9 Cu showed higher amount 0.176 ppm in the liver. In 2007-8 and 2008-9, both years Cu was showing 0.062 and 0.061 ppm high concentrations in the brain. In 2007-8 and 2008-9, both years Cu was found 0.540 and 0.531 ppm concentration in the heart as high level as compared to other three metals. The allowable value of copper for human is 10mg/kg. Lasbella, Liaquatabad, Grumandir and Empress Market are areas of station B, and all areas are residential except few small industries at Liaquatabd.

Samples of station C showed high level of Cu concentrations in the liver 2.524 and 2.490 ppm in year 2007-8 and 2008-9, respectively. In 2007-8 and 2008-9, both years Cu showed 0.064 and 0.062 ppm high concentrations in the brain. In 2007-8 and 2008-9, both years Cu was found high concentrations 0.848 and 0.968 ppm in the heart as high level as compared to other three metals. The allowable value of copper for human is 10mg/kg. Statistical analysis also confirmed that the liver is highly concentrated by all metals with a wide 95% confidence band. Malir, Landhi and Korangi Industrial areas were included in industrial area of Karachi and have a lot of industrial pollution. Some studies already reported different types of pollution in this area including ECD's contamination (Khan *et al*., 2009). Based on present findings, station C was found to be the most contaminated area compared to all five other stations

Table 9. 95% Confidence interval for trace metals concentration (ppm), Liver,heart and brain of *Gallus domesticus* at Station A-F from June 2007- May 2008.

	Ograns	**Metals**			
		Cd	**Ni**	**Cu**	**Pb**
Station A	Liver	0.104 ± 0.006	0.023 ± 0.002	0.767 ± 0.003	0.060 ± 0.004
	Brain	0.105 ± 0.005	0.320±0.0004	0.050 ± 0.007	0.040 ± 0.001
	Heart	0.113 ± 0.005	0.092 ± 0.003	0.161 ± 0.003	0.036 ± 0.003
Station B	Liver	0.198 ± 0.007	0.007 ± 0.001	0.142 ± 0.002	0.010 ±0.0008
	Brain	0.002 ±0.0008	0.002 ±0.0007	0.060 ± 0.001	0.046 ± 0.003
	Heart	0.009 ±0.0006	0.015 ± 0.001	0.539 ±0.0007	0.049 ± 0.001
Station C	Liver	1.020 ± 0.001	1.012 ±0.0004	2.522 ± 0.001	1.054 ± 0.002
	Brain	0.003 ± 0.001	0.001 ±0.0004	0.061 ± 0.002	0.051 ± 0.001
	Heart	0.172 ± 0.003	0.120 ± 0.001	0.846 ± 0.002	0.161 ± 0.003
Station D	Liver	0.180 ± 0.003	0.022 ± 0.004	0.529 ± 0.005	0.059 ± 0.001
	Brain	0.001 ±0.0004	0.001 ±0.0003	0.051 ± 0.015	0.034 ± 0.002
	Heart	0.075 ± 0.001	0.021 ± 0.002	0.668 ± 0.042	0.068 ± 0.001
Station E	Liver	0.039 ± 0.002	0.016 ± 0.002	0.128 ± 0.002	0.078 ± 0.002
	Brain	0.001 ±0.0000	0.001 ±0.0000	0.051 ± 0.001	0.031 ± 0.001
	Heart	0.047 ± 0.003	0.015 ± 0.002	0.547 ± 0.001	0.061 ± 0.006
Station F	Liver	0.211 ± 0.004	0.024 ± 0.001	0.541 ± 0.002	0.070 ± 0.002
	Brain	0.001 ±0.0003	0.001 ± 0.001	0.035 ± 0.002	0.041 ± 0.001
	Heart	0.068 ± 0.001	0.022 ± 0.002	0.051 ± 0.011	0.083 ± 0.003

In 2007-8 and 2008-9, both years Cu was showing 0.069 and 0.065 ppm high concentrations in the brain, respectively. In 2007-8 and 2008-9, both years Cu was found 0.702 and 0.621 ppm concentration in the heart, respectively, as high level as compared to other three metals. Station D have a Garden, Lyari and Lee Market areas and all areas are polluted.

At the station E, Copper was shown to have high level concentrations in the liver 0.132 and 0.428 ppm during 2007-8 and 2008-9, respectively. In 2007-8 and 2008-9, both years Cu was showing 0.052 and 0.056 ppm high concentrations in the brain, respectively. In 2007-8 and 2008-9, both years Cu was found 0.548 and 0.714 ppm concentration in the heart, respectively, as high level as compared to other three metals. The Hyderabad city area is a mainly residential area, and all samples were shows high concentration of Cu as compared to other three metals.

At the station F, Copper was shown high level concentrations in the liver 0.546 and 0.542 ppm during 2007-8 and year 2008-9, respectively. In 2007-8 Pb was shown 0.042 and 0.065 ppm high concentrations in the brain, while in year 2008-9 high concentrations of Cu 0.042 and Pb 0.042 ppm were found, respectively. In the year 2007-8 high concentration of Pb 0.089 ppm was found in the heart, while in 2008-9, concentration of Cd 0.070 and Pb 0.070 ppm were recorded, respectively, as high level as compared to other three metals. The allowable value of Lead for human is 1mg/kg (Anonymous, 2000).

Thatta city has one cement factory, while other areas consist of agricultural land and residential areas and some samples had the highest concentration of Pb as compared to other three metals. Lead can induce a wide range of adverse effects in humans depending on the dose and duration of exposure. The toxic effects range from inhibition of enzymes to the production of severe pathology or death (Goyer, 1990). Zalewski (2003) reported lead concentration for the mallards was from 0.050 mg/kg to 0.892 mg/kg (mean 0.225 mg/kg) in the liver and 0.038 mg/kg to 0.283 mg/kg in breast muscles. In the present study, concentrations of Pb were found in brain 0.042 and 0.065 ppm in the samples of 2007-8, and 0.089 ppm in the year 2008-9.

The results of this study show that samples of all stations were indicated high concentration of copper as compared

Table 10. 95% Confidence interval for trace metals concentration (ppm) Liver,heart and brain of *Gallus domesticus* at Station A-F from. June 2008- May 2009.

Metals					
	Organs	**Cd**	**Ni**	**Cu**	**Pb**
Station A	Liver	0.199 ± 0.003	0.022 ± 0.000	0.168 ± 0.005	0.009 ± 0.011
	Brain	0.126 ± 0.005	0.297 ± 0.002	0.040 ± 0.001	0.041 ± 0.002
	Heart	0.110 ± 0.003	0.067 ± 0.004	0.147 ± 0.001	0.040 ± 0.001
Station B	Liver	0.141 ± 0.002	0.001 ± 0.001	0.171 ± 0.003	0.008 ± 0.001
	Brain	0.003 ± 0.001	0.002 ± 0.001	0.057 ± 0.002	0.040 ± 0.000
	Heart	0.009 ± 0.001	0.009 ± 0.001	0.526 ± 0.004	0.055 ± 0.001
Station C	Liver	1.016 ± 0.001	1.019 ± 0.001	2.486 ± 0.003	1.061 ± 0.002
	Brain	0.005 ± 0.001	0.001 ± 0.000	0.061 ± 0.001	0.040 ± 0.001
	Heart	0.177 ± 0.002	0.120 ± 0.001	0.961 ± 0.005	0.163 ± 0.001
Station D	Liver	0.172 ± 0.005	0.027 ± 0.005	0.594 ± 0.005	0.055 ± 0.007
	Brain	0.005 ± 0.001	0.001 ± 0.000	0.055 ± 0.005	0.036 ± 0.002
	Heart	0.068 ± 0.001	0.020 ± 0.001	0.600 ± 0.008	0.082 ± 0.006
Station E	Liver	0.040 ± 0.001	0.021 ± 0.001	0.400 ± 0.014	0.096 ± 0.006
	Brain	0.001 ± 0.000	0.001 ± 0.000	0.050 ± 0.002	0.039 ± 0.001
	Heart	0.069 ± 0.003	0.022 ± 0.001	0.684 ± 0.017	0.062 ± 0.001
Station F	Liver	0.211 ± 0.003	0.028 ± 0.003	0.539 ± 0.002	0.056 ± 0.006
	Brain	0.001 ± 0.000	0.003 ± 0.002	0.041 ± 0.001	0.039 ± 0.002
	Heart	0.069 ± 0.001	0.022 ± 0.001	0.051 ± 0.001	0.068 ± 0.002

to other three metals and mostly metals transferred through poultry feeds because shop areas have not been contributed heavy metal contamination (normally shops hold chickens one and two days in his shop before selling) and according to present findings, the major source of metal contamination could be from poultry feed in all six stations.

Feed samples collected during 2007-8 from all stations, were found to have concentrations ranged of Cd 0.120, Ni 0.312, Cu 0.178 and Pb 0.040 ppm. While in year 2008-9, samples were found an amount of Cd 0.128, Ni 0.88, Cu 0.168 and Pb 0.045ppm. Based on feeding samples results, metals may have been transferred via poultry feeds to poultry chickens, because the feed processing system has not been according to standards and very unhygienic conditions has been observed during the study.

STATISTICAL ANALYSIS

For statistical analysis, statistical packages for social sciences (version 13) had been used for the analysis of trace metals concentration. A total of 144 observations was studied and the following results were noted.

It is observed that the liver is more concentrated with Cu at all stations, while Ni is almost less concentrated in all organs. It is also observed that all metals are very less concentrated in the brain or in other words the 95% confidence interval shows a narrow band for both sampling periods. At station C (Malir, Landhi, Korangi Industrial area), it is observed that the liver is highly concentrated by all metals with a wide 95% confidence band. All trace metals are randomly correlated to each other. For example at station B (Lasbella, Liaquatabad, Grumandir, and Empress Market), it is observed that Cd and Ni, Cd & Cu and Cd & Pb have no linear association in chicken heart, while at station C, Ni & Pb have no linear association in chicken liver. One interesting thing is noted here that there exists a strong negative relationship between Cd and Cu in brain from station C (Malir, Landhi, Korangi Industrial area), while strong positive in station F (Thatta city).

Table 11. Correlation between Trace metals concentration (ppm), Jun 2007- May 2008.

	Correlation between Trace metal concentrations						
	Organs	ρ_{CdNi}	ρ_{CdCu}	ρ_{CdPb}	ρ_{NiCu}	ρ_{NiPb}	ρ_{CuPb}
Station A	Liver	-0.477	0.655	-0.109	-0.913	0.520	-0.469
	Brain	0.209	-0.090	-0.164	0.891	0.386	0.514
	Heart	0.248	-0.413	-0.388	-0.284	-0.580	-0.050
Station B	Liver	-0.354	-0.321	0.217	0.183	-0.203	-0.049
	Brain	0.664	0.103	0.264	-0.079	0.844	-0.195
	Heart	0.000	0.000	0.000	-0.259	0.116	-0.211
Station C	Liver	0.193	-0.464	-0.478	-0.079	0.000	0.651
	Brain	-0.253	-0.909	0.020	0.162	0.534	-0.051
	Heart	-0.370	0.361	0.201	-0.186	-0.647	0.000
Station D	Liver	0.241	0.497	0.398	0.521	0.471	-0.089
	Brain	-0.135	0.544	-0.313	-0.263	-0.042	-0.711
	Heart	-0.467	-0.877	0.000	0.465	0.187	0.201
Station E	Liver	0.678	-0.493	-0.300	-0.502	-0.456	0.812
	Brain	-0.135	0.290	0.408	-0.391	-0.233	0.706
	Heart	-0.589	-0.394	0.050	0.672	-0.253	-0.092
Station F	Liver	0.465	-0.317	0.015	-0.177	0.218	0.325
	Brain	0.225	0.864	-0.600	-0.121	-0.676	-0.420
	Heart	-0.330	0.519	-0.317	-0.346	0.421	-0.465

Comments: There is no correlation between Cd and Ni, Cu and Pb in the Heart of Chicken at station B . There is no correlation between Ni and Pb in the Liver of Chicken at station C

In this study, general linear model has been applied based on data for a sampling period from June 2007 to May 2009. Four levels of metals, six levels of stations and three levels of organs are being observed. And it was noted that all the hypotheses of interests are rejected and concluded that all metals, stations and organs are significantly different (see Tables 7-12).

Linear regression analysis has been applied for the said sampling period and it was observed that the regression model can only explain 6.1% variation during Jun 2007 to May 2008 and only 4.7% variation during Jun 2007 to May 2009 of the control variable. And also the analysis of variance of the regression parameter shows insignificant. It is concluded that regression model is not suitable for the metal concentration.

Overall brain has recorded very little amount of metals irrespective by stations, while Cu is more influential metal present on all organs with a significant amount. Station C (Malir, Landhi, Korangi Industrial area) has higher concentration of trace metals than other stations, because Landhi and Korangi industrial areas are the polluted areas of Karachi city.

CONCLUSION

The risk associated with the exposure to heavy metals present in food product had aroused widespread concern in human health. Chicken is an important source of protein in Pakistan and widely consumed in all four provinces. This study has concluded that all chickens samples collected from Karachi, Hyderabad and Thatta city were contaminated with Cadmium (Cd), Nickel (Ni), Copper (Cu) and Lead (Pb). Whereas Copper contamination was found to be the highest compared to other metals tested. Based on poultry feed samples' testing during the present study, metals could have been transferred via poultry feeds to poultry chickens. Observations made in the Karachi poultry feed processing facility during 2007-2009 showed unhygienic environment in the preparation of poultry feed. It is recommended that the government of Sindh adopts a general procedure for control of contamination in the poultry feed facilities/Mills.

Table 12. Correlation between trace metals concentration (ppm), Jun 2008- May 2009.

	Correlation between Trace metal concentrations						
	Organs	ρ_{CdNi}	ρ_{CdCu}	ρ_{CdPb}	ρ_{NiCu}	ρ_{NiPb}	ρ_{CuPb}
Station A	Liver	-0.253	-0.296	-0.303	0.012	-0.151	0.689
	Brain	-0.129	0.547	-0.344	-0.309	0.057	-0.598
	Heart	-0.731	0.438	0.352	0.007	-0.409	-0.241
Station B	Liver	-0.027	0.483	0.335	0.576	0.202	0.302
	Brain	0.644	-0.665	0.232	-0.349	0.190	-0.276
	Heart	-0.351	0.414	-0.251	0.195	0.504	0.303
Station C	Liver	0.420	0.265	0.224	-0.362	-0.611	0.698
	Brain	0.677	-0.313	0.573	-0.174	0.741	-0.645
	Heart	0.148	-0.501	0.232	-0.682	0.576	-0.332
Station D	Liver	-0.054	0.352	-0.035	-0.391	0.042	-0.544
	Brain	-0.234	0.186	0.405	0.217	0.186	-0.213
	Heart	0.686	-0.185	0.035	-0.300	-0.390	0.081
Station E	Liver	0.828	0.861	-0.819	0.880	-0.911	-0.973
	Brain	0.158	0.394	-0.304	-0.050	0.480	-0.344
	Heart	0.391	-0.126	-0.380	-0.534	-0.151	-0.024
Station F	Liver	0.574	-0.590	-0.305	-0.328	0.025	0.100
	Brain	0.435	0.480	-0.653	0.647	-0.623	-0.721
	Heart	0.486	-0.341	0.386	0.254	-0.356	-0.757

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