EXPOSURE AND EFFECTS OF TOXIC TRACE METALS IN BIRDS OF PAKISTAN

Sana Ashraf[1], Naeem Akhtar Abbasi[1*], Sajid Rashid Ahmad[1]

[1] College of earth and environmental sciences, university of the Punjab, Quaid-e-Azam campus, Lahore
* Corresponding author E. mail: abbasi_akhtar4045@yahoo.com

ABSTRACT: Increasing concentration of trace metals in the environment and their subsequent health effects in living organisms has become a major threat particularly in developing parts of the world. Therefore present study was designed to investigate the concentration of trace metals such as Pb, Cd, Cr and Cu in liver, pectoral muscle, pelvic muscle and blood of two terrestrial (Bank Myna; *Acridotheres ginginianus* and Jungle Babbler; *Turdoides striata*) and two aquatic (Grey Heron; *Ardea cinerea* and Cattle Egret; *Bubulcus ibis*) bird species collected from the premises of Lahore, Pakistan. The detection frequency of trace metals in collected samples were highest in Cd (100%) followed by Pb (83%), Cr (80%) and Cu (76.5%). Species revealed heterogeneous levels of metals in all organs. In general the mean concentration (µg/g) of trace metals in organs and blood of terrestrial species followed the pattern as Pb > Cu > Cr > Cd contrary to aquatic species in which the trend was Cu > Pb > Cr > Cd. Highest concentration of Pb (3.23 µg/g) was reported in pelvic muscle of jungle babbler, Cd (0.15 µg/g) in liver of bank myna, Cr (0.40 µg/g) in pelvic muscle of Cattle Egret and Cu (1.89 µg/g) in liver of Cattle Egret. Whereas lowest concentration of Pb (0µg/g) and Cd (0.09 µg/g) was reported in blood and pelvic muscle of Grey Heron respectively, Cr (0µg/g) in blood of Bank Myna, Grey Heron and Cattle Egret, Cu (0 µg/g) in blood of Grey Heron, Cattle Egret and Jungle Babbler. Concentration of metals varied significantly ($P<0.05$) among organs however, no significant difference ($P>0.05$) was observed among species except Cu($P<0.05$). Further, no significant difference ($P>0.05$) of metals was observed between feeding guilds and habitat of the species. The increasing concentration of toxic trace metals in the birds reflects deteriorating environmental health as a result of greater metals exposure which must be reduced through proper legislation and strict implementation of laws.

Key words: Toxic trace metals, Bioaccumulation, Bio-indicator, Pakistan, Bird.

(Received 12.10.2022 Accepted 22.11.2022)

INTRODUCTION

As a result of development and modern procedures enormous quantities of contaminants have ceaselessly been brought into biological systems. Trace metals are high density elements which are non-biodegradable in nature. A portion of these substantial metals are very essential forever however just if present in little amount as components, for example, Fe, Cu and Zn (Hosseini et al., 2015). Trace metals are industrious contaminants that bio-accumulate in natural ways of life, winding up progressively, hazardous to human and untamed life (Swaileh & Sansur, 2006). Heavy metals are pervasive, exceedingly constant, and non-decomposable with extensive bio-coherent half-life (Burger et al., 2007).The sources of heavy metals in both terrestrial and oceanic environments are unintended omission, squander discard and accidental leakage. Heavy metals bio-magnify in the cells of animals due to successive vulnerability of these metals (Malik et al., 2014).Due to poisonous nature of heavy metals, national interest is increasing day by day to avoid heavy metals contamination. So, it is very important to survey, screen, assess, oversee and rectify environmental harm (Movalli, 2000; Naccari et al., 2009).Living beings fluctuate in their degree of metal accumulation in a living biological system, relying on their eating routine, trophic dimension, age, size, gender and different other inner or outer factors. To measure the amount of metals in complete sections of the environment is troublesome, requires more time period and efforts. That's why , those species are used to estimate the amount of trace metals which are susceptible to environment and could highlight the condition of an ecosystem in a broader way (Malik et al.,2014). We can gain data over a vast region throughout each sampling site with the help of animals that are at the highest point of the food chain. We can gather information on bioavailability as well as by what method, location and at what time heavy metals are exchanged in a food chain (Battaglia et al., 2005).To determine general health of environment use of birds as bio-indicator species is very effective (Hofer, Gallagher, & Holzapfel, 2010).Birds are more vulnerable to heavy metals as they have high metabolism rates. So heavy metals can easily absorbed into their gastrointestinal tract from atmospheric air (Szymczyk & Zalewski, 2003).Since birds are bounteous, generally conveyed, enduring, touchy to climatic natural changes, are high on the evolved way of life and sensible to estimate bioaccumulation in organs to assess deadly and sub-deadly impacts. That's why they are broadly use as bio-indicators (Naccari et al., 2009).Currently usage of living organisms such as sentimental species provides more accurate results as compared to chemical and physical analysis to detect the heavy metals. This provides us exact information of bio-accessibility and bio transference of contaminants and we can gain some physiological and conduct signs of instigated poisonous quality (Roux & Marra, 2007).The sources of heavy metals are air, water or food through which birds are susceptible to heavy metals. The metal can be deposit, accumulate or it can be discharge, once it has entered the body of birds (Burger, 1993). Sentimental species release heavy metals via excreta or through settling them in the preen gland, salt gland (Dauwe et al., 2000) and plumes (Burger, 1993). Eggs are source of elimination of heavy metals in female birds (Dauwe et al., 1999).There are two ways through which birds are susceptible to heavy metals either by externally through physical contact or internally through eating the poisonous food (Jarosław & Ban, 2013). Birds are susceptible to heavy metal externally either by deposition of the metals on the feathers from the environment or via cleaning of its feathers (Dauwe et al., 2003).Moreover trace metals are integrated in the keratin structure of birds during feathers development via absorption of metals by food as feathers are attached with the bloodstream (Tsipoura et al., 2008). In food chain the range of accumulation of metals increase with the age of living organisms as me move upward (Van Straalen & Ernst, 1991).Numerous factors are responsible for the intake and accumulation of trace metals in avian species which include age, diet and metabolism of the exposed birds (Boncompagni et al., 2003).

Living Organisms like birds are affected by different toxic agents. For instance, metals increases the violent performance in regional birds, reduce functionality of immune system, increase vulnerability to illnesses, anxiety and cause fluctuations in communicative patterns (Swaileh & Sansur, 2006).Different neurological and physical problems such as reduced avian grasp sizes and fecundity, conceiving failure, burrowing death (Burger, 1993), social defects of chicks (Burger & Gochfeld, 1998), reduction in birds body bulk and detained fledge time (Jansenns et al., 2003) are caused due to bioaccumulation of metals in birds.In Pakistan and china Different studies have conducted on European black vulture and blue rock pigeon. It is very important to estimate heavy metals in various tissues and organs for assessment at population level, because there are variations between the concentrations of similar metal in different tissues of birds (Vashishat & Kler, 2014).It is very important to regularly evaluate the toxic metals in different organs of birds. It will help to observe the pollution in an area, province or a country. The metals bio-accumulate in the food chain when organism is incapable of removing metal from its body(Szymczyk & Zalewski, 2003). The main objectives of the study are: 1.To determine the concentration of the trace metals (Pb, Cd, Cr and Cu) in Liver, Pectoral muscle, Pelvic muscle and blood of birds. 2. To investigate the comparison of trace metals bioaccumulation between terrestrial and aquatic species of birds. 3. To investigate the comparison of trace metals bioaccumulation between different feeding guilds of birds species. 4. To elucidate the difference of metals accumulation among species as well as among various organs of species.

MATERIAL AND METHODS

Study Area: Samples were collected from Manga Mandi, 45 km from Lahore, Pakistan. The coordinates of Lahore are 31º 34' 3620" North and 74º 19' 45.7536" East. Lahore is center of Punjab and second largest city of Pakistan. Lahore is heart of Pakistan. Total population of Lahore is 11.13 million. It is one of the most populous cities of Pakistan. Lahore has semi-arid climate. June is hottest month of Lahore with a temperature of 40º whereas January is coolest month of the year. Fog remains thick in January. The annual monsoon rainfall Lahore receives is 24.76 inch. Main crops of the area sugar cane, rice, maize and cotton. Main fruits are Citrus, guava and mango. Whereas main vegetables are potato, onion, turnip and cauliflower. Lahore is surrounded by many industries. Anthropogenic activities are major source of pollution in Lahore. The center of Punjab is one of the most contaminated city around world. It is between the 10 most polluted cities in world. The major sources of contamination are industries, heavy traffic, solid waste issues and many more. The impacts on animals are visible. The wastewater from many industries is released in River Ravi. The river is now polluted due to discharge of effluents. The sludge settled in the river.The geographical coordinates of Manga Mandi are 31º 18' 31" North and 74º 3' 5" East. It is an industrial area with diverse population and involve anthropogenic activities. The climate of the area is hot in summers and cold in winter. A Little rainfall is observed throughout the year. Average temperature is 24.2º. June is one of the hottest month of the year with an average temperature of 34º. Whereas January is the coolest month with an average temperature of 12.2º.

Species selection and sample preparation: A total of 16 species of birds were collected from Manga Mandi, 45 Km from Lahore in March-April 2019. These species belong to four different families Ardeidae (Cattle Egret); sturnidae (Bank Myna); Ardeidae (Grey Heron); Leiothrichidae (Jungle Babbler) and four different feeding guilds viz. omnivorous (Bank Myna), carnivorous (Grey Heron) , invertebrate predator (Cattle Egret) and granivorous (Jungle Babbler).Besides the species were categorized as terrestrial (Bank Myna, Jungle Babbler) and aquatic species (Grey Heron, Cattle Egret) based on their habitat .This study will provide the chance to approximate the heavy metals amount in alike and dislike species within same location.The present investigation was conducted on three species of Cattle Egrets weighted 332-368 g, three species of Grey Heron weighted 290-294g, five species of Bank Myna weighted 78-88g and five species of Jungle Babblers weighted 73-85g.

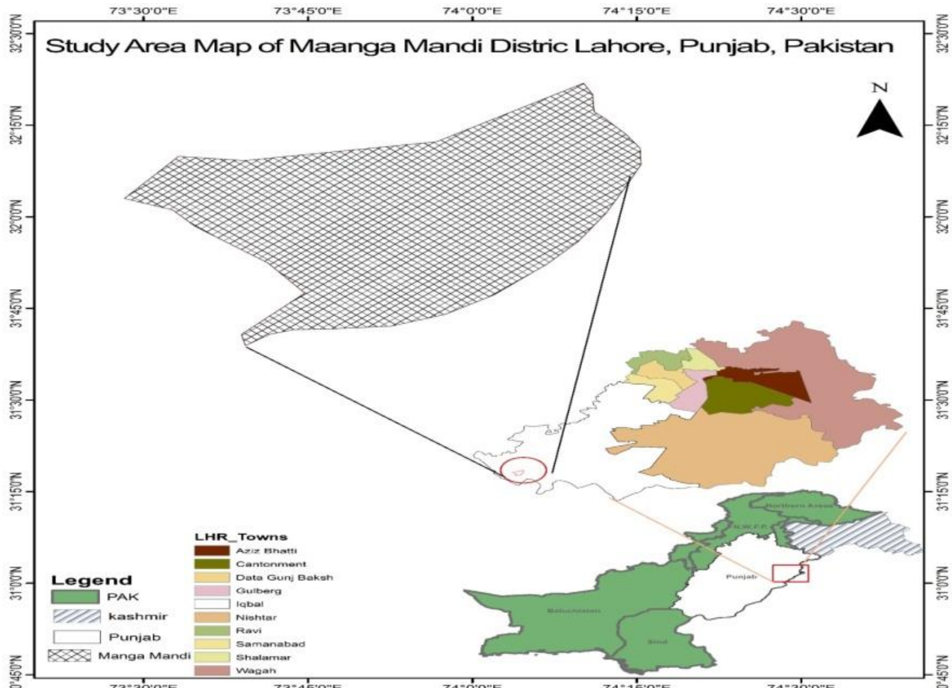

Fig.1.Map of Manga Mandi, District Lahore, Punjab, Pakistan –sampling site for birds' collection.

The samples were collected from the birds captured in the framework of some other study. Birds were slaughtered, place in zipper plastic bags and stored in freezer for advanced analysis. The birds were weighted and its length is measured with the help of measuring scale. In order to analyze the organ and tissue parts, dissection of birds was done. Different parts such as liver, pectoral muscles, pelvic muscles were obtained. Blood was also obtained for analysis.All the specimens were preserved in plastic bags. Storage of blood was done in properly labeled EDTA tubes. All the samples of tissues and blood were stored in refrigerator at -4 degrees Celsius temperature. All dissection and storage was carried out in Toxicology Lab of CEES, University of the Punjab.

Trace metals Analysis: Trace metals (Cd, Pb Cr and Cu) were analyzed in bird tissues as well as in blood by using Atomic Absorption Spectrometer. For this purpose digestion of samples was done.

Reagents: Nitric Acid (HNO_3) and Per-chloric Acid ($HCLO_4$) used in the digestion was of ultra-pure quality. Hydrogen Peroxide (H_2O_2) was also used for the removal of color. Distilled water was used for dilution and for washing all the glassware.

Digestion of bird tissues: Each dissected bird organ weighted on a weight machine and its wet weight was taken, after that it is then oven dried at 105°C.The dry sample then weight for its dry weight to determine the moisture content. The samples were then grind into powder form with the help of mortar and pestle. Forty eight samples of tissues (Liver, Pectoral and Pelvic muscles) were digested.For each tissue (n=48) the digestion process was conducted by using 0.5g of chopped tissue sample. 6ml HNO_3, 4ml H_2O_2 and 2ml $HCLO_4$ was used to digest the sample. Hot plate was used to speed up the digestion process.First of all 0.5g of sample was taken in a beaker. In the beaker containing sample, 6ml HNO_3 and 2ml $HCLO_4$ was added and heated the solution in hotplate. The temperature was kept at 85°C. After 1-2 minutes 4 ml H_2O_2 was added to remove the color. Digestion was perused till there are no fumes appeared in solution and a colorless solution appeared and allowed it to cool.The sample was then transferred into a measuring flask and distilled water is added to make the volume up to 25 ml. Then filtration of sample was done with whatman filter paper#42 to remove the precipitate that formed and stored in the properly labeled sampling bottles (Gebremedhin & Berhanu, 2015).

Digestion of blood samples: Each blood sample of bird (n=16) was digested. For this purpose 0.5ml of each blood sample was taken into a test tube which is accurately labelled. The blood was transferred from EDTA tube with the help of syringe into test tube.For each blood sample two acids HNO_3 and H_2O_2 in 2:1 v/v were used. Each test tube containing three millimeters of these two acids and 0.5 ml blood samples of each bird was kept at room temperature for almost 10 min.The mixture containing blood and acids was warmed up at 85°C.A clear and transparent solution was obtained. The blood samples were digested. This whole process of digestion needed 1-2 min.The solution obtained was cooled. Distilled water was added by using measuring flask and digested sample was brought up to the volume of 25ml. The sample solutions were filtered to eliminate precipitate that formed and stored in properly labeled sampling bottles. Whatman filter paper # 42 was used to remove precipitate. Each 25ml filtered sample solution was placed in properly labelled plastic bottle. Now the samples were ready to run in Atomic Absorption spectrometer for determining heavy metals.

Procedure for Atomic Absorption Spectrophotometer: Atomic absorption spectrophotometer (AAS) model: A Analyst 800, Perkinelmer present in College of Earth and Environmental sciences was used. Blank solutions were used to calibrate the spectrophotometer. Firstly, standard solutions were run in spectrophotometer to analyze the trace metals (Pb, Cd, Cr, and Cu). After standard solution, digested sample solution were run. Respective metal lamps were used to measure the amount of metals in sample. Four Reagent blanks were run with sample solution as standard reference material for respective metal. For the purpose of quality control duplicate and spiked samples were also run in AAS. The same chemicals which we used for digestion of sample were present in reagent blanks plus the water for the dilution of blank.

Statistical Analysis: Statistical software (IBM SPSS statistics version 21) was used to perform statistics tests. Statistics applied included calculation by basic statistics for example mean and standard deviation (SD) by using Microsoft Excel. Graphs were also prepared by using the same software. To find mean values descriptive statistics was used. Two way ANOVA was applied to determine the significant difference of species of birds, organs and their interaction on heavy metal concentration. Significant difference between various species and tissues were estimated by heavy metal concentration at confidence interval of 95%. One way ANOVA was used to compare the accumulation between species based on feeding groups and habitat. Pearson co-relation was used to estimate the relationship between metals in different species. Map was generated using Google Earth and ArcGIS.

RESULTS AND DISCUSSION

Percentage Detection Frequency of Trace Metals in the bird Samples: The detection frequency of Cadmium is 100% means detected in all samples (Figure 2). However Chromium detection frequency is 80%, Lead is 83% and of copper is 76.5%. Means Pb, Cu and Cr are not detected in all of the bird tissues and organ samples. Some organs and blood did not indicate any concentrations of Pb, Cu and Cr in them.

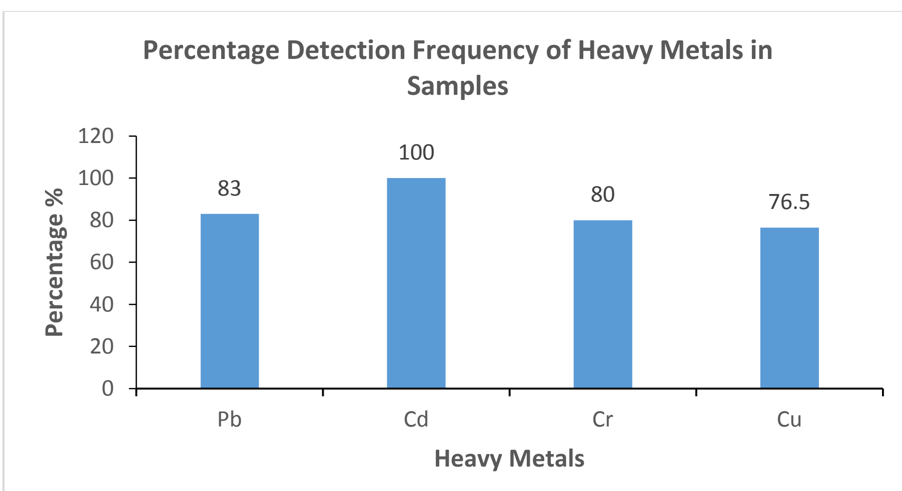

Fig. 2. Percentage Detection Frequency of trace metals in samples of birds

Heavy Metal profile of selected organs and blood of birds: Four heavy metals (Pb, Cd, Cr and Cu) were analyzed in 64 samples of birds to investigate the concentration of these metals in organs and blood. Accumulation of heavy metals in different tissues and blood was heterogeneous. Table 1 shows different mean ± standard deviation values and range of trace metals (Pb, Cd, Cr and Cu) in liver, pectoral muscle, pelvic muscle and blood of birds. Highest mean concentration of Cu (1.82±0.03) was reported in liver of Cattle Egret (*Bubulcus ibis*) followed by pectoral muscle, pelvic muscle and blood. The highest concentration of Cd (0.17±0.08) was observed in liver of Bank Myna (*Acridotheres ginginianus*). The accumulation pattern of Cd in Bank Myna was of following order: liver > pectoral muscle > pelvic muscle > blood. Whereas liver of Jungle Babbler (*Turdoides striata*) accumulated the maximum concentration of Cr. The sequence of chromium concentration in Jungle Babbler was: liver > pectoral muscle > pelvic muscle > blood. The highest mean concentration of Pb (3.23±0.71) was reported in pelvic muscle of Jungle Babbler (*Turdoides striata*).

Table 2 shows that the significant difference between organs is less than 0.05 means they are statistically significant. In species Cu is statistically significant ($P<0.05$) whereas in species the Pb, Cd and Cr are not statistically significant as $P>0.05$. So, concentration of heavy metals influence organs of birds. No statistically significant difference was observed in Pb, Cd, Cr in case of species and organ interaction except Cu in which ($P<0.05$).

Comparison of trace metal concentration between organs and species: Trace metals showed heterogeneous concentration in different bird organs. In general maximum concentration of Pb and Cu was reported in all species. Trace metal concentration in some samples of blood were not detected. The highest concentration of Cu (1.89 µg/g) wasestimated in liver of cattle egret whereas lowest concentration of Cr and Cu were reported in blood of cattle egret.The highest amount of Pb (0.99 µg/g) was observed in pelvic muscle of jungle babbler.

Figure3 shows that the highest lead concentration is reported in organs of Bank Myna (*Acridotheres ginginianus*) followed by copper. The lead in organs follow this sequence: pectoral muscle > liver > pelvic muscle > blood. Whereas the sequence of copper in organs is pectoral muscle > pelvic muscle > liver > blood. The concentration of other metals are not as significant as lead and copper. The overall trace metal concentration is in following order: Pb > Cu > Cr > Cd.Figure 4 shows that the highest metal concentration in organs of Grey Heron (*Ardea cinerea*) is of copper followed by lead. The copper in organs follow this sequence: liver > pectoral muscle > pelvic muscle > blood. The sequence of lead in organs is same as copper. The concentration of other metals are not as significant as lead and copper.Figure 5 shows that the highest metal concentration in organs of cattle Egret (*Bubulcus ibis*) is of copper followed by lead. The copper in organs follow this sequence: liver > pectoral muscle > pelvic muscle > blood. The sequence of lead in organs is liver > pelvic muscle > pectoral muscle > blood. The concentration of other metals are not as significant as lead and copper. The overall trace metal concentration is in following order: Cu > Pb > Cr > Cd.Figure 6 represents that the highest lead concentration is in organs of Jungle babbler (*Turdoides striata*) followed by copper. The lead in organs follow this sequence: pelvic muscle > liver > pectoral muscle > blood. Whereas the sequence of copper in organs is pelvic muscle > pectoral muscle > liver > blood. The concentration of other metals are not as significant as lead and copper. The overall trace metal concentration is in following order: Pb > Cu > Cr > Cd.

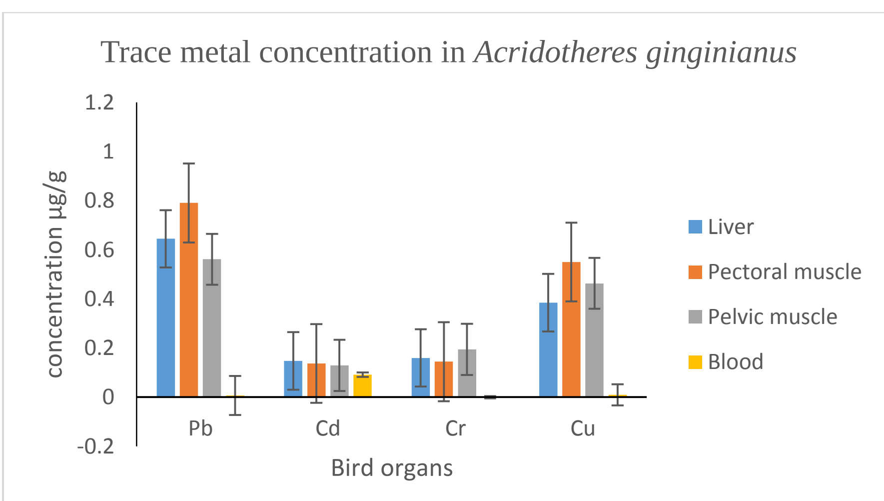

Fig. 3.Trace metal concentration in different organs and blood of Bank Myna (*Acridotheres ginginianus*).

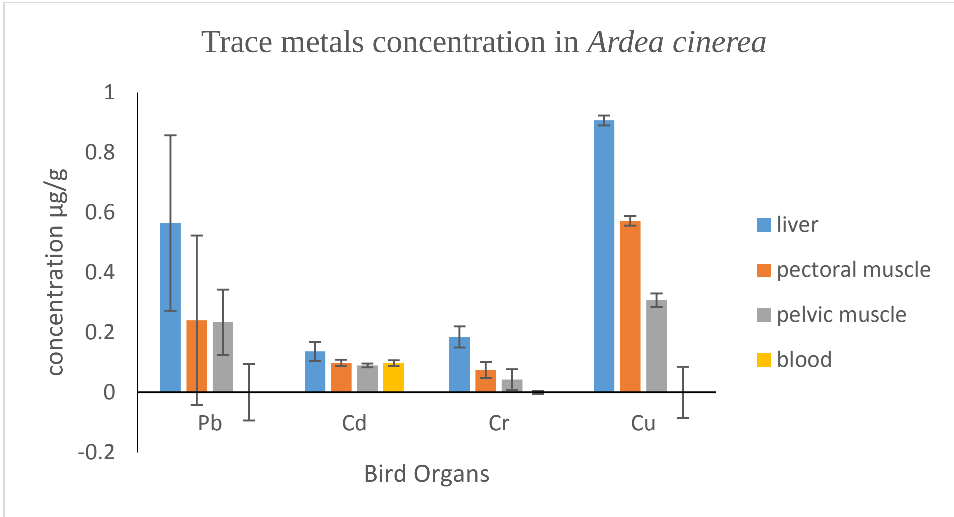

Fig. 4.Trace metal concentration in different organs and blood of Grey Heron (*Ardea cinerea*)

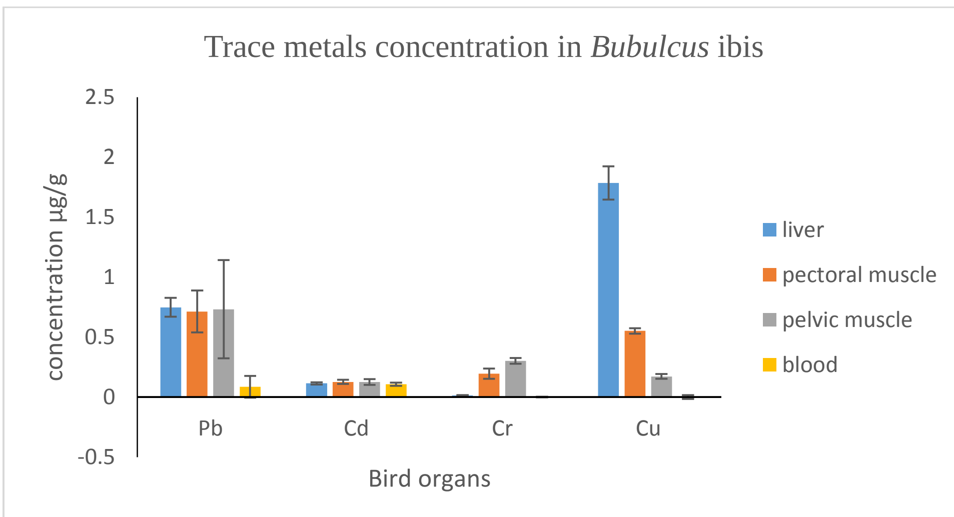

Fig. 5Trace metal concentration in different organs and blood of cattle Egret (*Bubulcus ibis*)

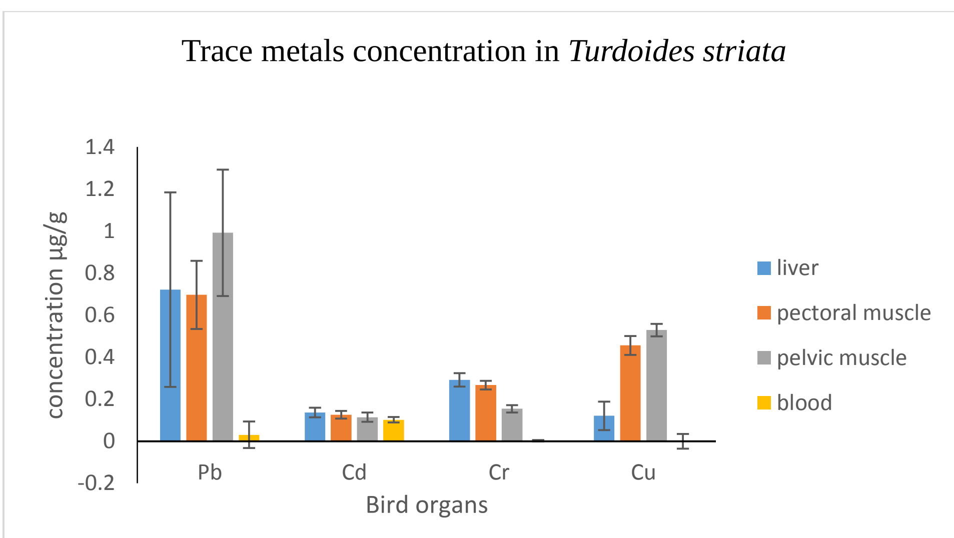

Fig. 6. Trace metal concentration in different organs and blood of Jungle babbler (*Turdoides striata*)

Table 1. Mean ± standard deviation (µg/g) and Range (µg/g)of heavy metals in different organs and blood of four species of birds.

Species	*n*	organs	Pb	Cd	Cr	Cu
			Mean±S.D. (Min-Max)	Mean±S.D. (Min-Max)	Mean±S.D. (Min-Max)	Mean±S.D. (Min-Max)
Acridotheres ginginianus	5	Liver	0.64±0.25 (0.21-1.05)	0.15±0.03 (0.12-0.17)	0.16±0.03 (0.02-0.35)	0.38±0.01 (0.16-0.59)
		Pectoral muscle	0.79±0.18 (0.33-1.13)	0.14±0.02 (0.06-0.17)	0.14±0.04 (0.03-0.33)	0.55±0.09 (0.28-1.09)
		Pelvic muscle	0.56±0.32 (0.21-0.95)	0.13±0.03 (0.06-0.18)	0.19±0.03 (0.03-0.44)	0.46±0.03 (0.19-1.11)
		Blood	0.01±0.08 (0-0.03)	0.09±0.01 (0.08-0.10)	Not Detected	0.01±0.04 (0-0.05)
Ardea cinerea	3	Liver	0.56±0.29 (0.38-0.77)	0.14±0.03 (0.12-0.15)	0.18±0.04 (0.03-0.29)	0.91±0.02 (0.78-1.06)
		Pectoral muscle	0.24±0.28 (0.09-0.52)	0.1±0.01 (0.07-0.13)	0.08±0.03 (0.02-0.17)	0.57±0.02 (0.10-0.96)
		Pelvic muscle	0.23±0.11 (0.07-0.57)	0.09±0.01 (0.08-0.10)	0.04±0.03 (0-0.12)	0.31±0.02 (0.05-0.54)
		Blood	Not Detected	0.1±0.01 (0.09-0.10)	Not Detected	Not Detected
Bubulcus ibis	3	Liver	0.75±0.08 (0.13-1.98)	0.13±0.01 (0.11-0.13)	0.01±0.001 (0.01-0.02)	1.78±0.14 (1.64-1.89)
		Pectoral muscle	0.71±0.17 (0.18-1.00)	0.13±0.02 (0.09-0.16)	0.19±0.04 (0.03-0.35)	0.55±0.02 (0.17-1.03)
		Pelvic muscle	0.73±0.41 (0.48-0.88)	0.13±0.02 (0.12-0.14)	0.30±0.02 (0.22-0.40)	0.17±0.02 (0.11-0.24)
		Blood	0.09±0.09 (0-0.20)	0.11±0.01 (0.09-0.12)	Not Detected	Not Detected
Turdoides striata	5	Liver	0.72±0.46 (0.36-0.98)	0.14±0.029 (0.11-0.17)	0.29±0.03 (0.09-0.42)	0.12±0.07 (0-0.27)
		Pectoral muscle	0.69±0.16 (0.36-1.23)	0.13±0.02 (0.07-0.15)	0.27±0.02 (0.08-0.45)	0.46±0.05 (0.13-1.35)
		Pelvic muscle	0.99±0.3 (0.14-3.23)	0.11±0.02 (0.08-0.15)	0.15±0.02 (0.01-0.31)	0.53±0.03 (0.07-1.25)
		Blood	0.03±0.06 (0-0.13)	0.10±0.01 (0.10-0.11)	0.003±0.003 (0-0.01)	Not Detected

Table 2 Statistical significance of different species of birds, organs and their interaction on heavy metal concentration determined by two way ANOVA

Trace metals	**Species**		**Organ**		**Species*organ**	
	F value	P value	F value	P value	F value	P value
Pb	1.321	0.278	5.527	0.002	0.386	0.936
Cd	1.479	0.232	4.241	0.010	0.854	0.572
Cr	1.946	0.135	7.340	0.000	1.890	0.076
Cu	3.837	0.015	19.293	0.000	6.600	0.000

*P<0.05, significance level

Table 3 Comparison of trace metals concentrations (µg/g) among different Feeding Guilds (Omnivores, Carnivores, Invertebrate Predators and Granivores) and Habitat (Terrestrial/Aquatic) determined by ONE WAY ANOVA and LSD test for multiple comparisons.

Trace metals	Feeding guilds (Between groups)		Habitat (Aquatic/ Terrestrial) (Between groups)	
	F value	P value	F	P
Pb	1.121	0.348	0.999	0.321
Cd	1.237	0.304	2.144	0.148
Cr	1.287	0.287	1.727	0.194
Cu	1.512	0.221	3.438	0.068

Where*P<0.05, significance level

Comparison between different Feeding Guilds: In birds, accumulation of trace metals influenced by many factors such as trophic levels, taxonomic differences, age, gender and location. Most important factors that effects the accumulation of trace metals are feeding approach, type of food items and feeding habit of bird (Dauwe et al., 2000; Berglund et al., 2011).

In order to test the influence of trophic level in heavy metal accumulation birds were divided into Omnivore (Bank Myna), Carnivore (Grey Heron), invertebrate predator (Cattle Egret) and Granivore (Jungle Babbler).Table 3 shows the comparison of metal accumulation in various species of birds based on feeding guilds. No significant difference was observed (P>0.05) in species based on feeding guilds.

Comparison between terrestrial and aquatic species: In present study, trace metals mean concentration (µg/g) in tissues and blood of Bank Myna and Jungle Babbler (terrestrial species) followed the trend: Pb > Cu > Cr > Cd except blood of both species whereas in Cattle Egret and Grey Heron (aquatic species) the trend was following: Cu > Pb > Cr > Cd with exception of liver in Cattle Egret, pelvic muscle in Grey Heron and blood in both species. Table 4.3 reports that heavy metals are not statistically significant (P>0.05) between terrestrial and aquatic species.

Pearson Co-relation between metals in different bird species: In Bank Myna Pb is positively co-related with Cd and Cr. Cd is strongly positively co-related with Pb and Cr at P<0.01 (Table 4.4). In Grey Heron Pb is positively co-related with Cd at P<0.01 level whereas with Cr at P<0.05. Cd has positive co-relation with Pb and Cr. In Cattle Egret Cd and Cr are positively co-related at P<0.05 level. In Jungle Babbler Pb is positively co-related with Cr. whereas Cd has positive relation with Cr and negative with Cu. Cr is positively co-related with Pb and Cd. Cu has negative co-relation with Cd at P<0.05.Strong co-relation isobserved between the trace metals (Table 4). Toxic trace metals such as Pb, Cd and Cr influence each other strongly and have same source of origin.

Table 4. Co-relation among metals in Bank Myna, Grey Heron, Cattle Egret and Jungle Babbler

		Pb	Cd	Cr	Cu
Bank Myna	**Pb**	1			
	Cd	0.768**	1		
	Cr	0.719**	0.682**	1	
	Cu	0.344	-0.152	0.077	1
Grey Heron	**Pb**	1			
	Cd	0.658*	1		
	Cr	0.920**	0.651*	1	
	Cu	0.468	0.373	0.496	1
Cattle Egret	**Pb**	1			
	Cd	0.289	1		
	Cr	0.402	0.602*	1	
	Cu	0.205	-0.219	-0.385	1
Jungle Babbler	**Pb**	1			
	Cd	0.396	1		
	Cr	0.605**	0.736**	1	
	Cu	0.033	-0.549*	-0.122	1

*. Co-relation is significant at the 0.05 level (2-tailed)
**. Co-relation is significant at the 0.01 level (2-tailed)

DISCUSSION

Inter-specific difference of trace metals in organs and species: In liver of all species the concentration Cu (1.78 µg/g) was highest in Cattle Egret followed by Pb (0.75 µg/g) in Cattle Egret, Cr (0.29 µg/g) in Jungle Babbler and Cd (0.15 µg/g) in Bank Myna. In pectoral muscle of all species the concentration of Pb (0.79 µg/g) was maximum in Bank Myna followed by Cu (0.57 µg/g) in Grey Heron, Cr (0.26 µg/g) in Jungle babbler. Whereas in pelvic muscle of all species the accumulation of Pb (0.99 µg/g) was highest in Jungle Babbler followed by Cu (0.53 µg/g) in Jungle Babbler, Cr (0.30 µg/g) in Cattle Egret and Cd (0.13 µg/g) in Bank Myna. In the blood of all species, Cd (0.107 µg/g) was maximum in Cattle Egret. Whereas some trace metals were not detected in blood of different species**.**

The range of Pb was maximum (0.207-1.046 µg/g) in liver of bank myna whereas the range of Cd, Cu, and Cr was reported to be in following order: 0.119-0.172 µg/g, 0.024-0.352 µg/g,0.158-0.588 µg/g respectively. Trace metals in the liver of bank myna followed the trend Pb > Cu > Cr > Cd. Bank myna pectoral muscles contained highest amount of Pb (1.126 µg/g). The order of trace metals in pectoral muscle of bank myna was: Pb > Cu > Cr > Cd. Pelvic muscles of bank myna contained highest amount of Pb. The order of metals in pelvic muscle of bank mynawas Pb > Cu > Cr > Cd whereas blood of bank myna contained highest amount of Cd.The range of Cu (0.78-1.06 µg/g) was maximum in liver of Grey Heron. In liver, minimum mean concentration of Cd (0.14 µg/g) was reported in Grey Heron. Order of trace metals in liver of Grey Heron was following Cu >Pb > Cr > Cd. The highest concentration of Cu (1.89 µg/g) was reported in liver of cattle egret. Whereas lowest concentration of Cr and Cu was reported in blood of cattle egret.The Pb highest concentration (0.99 µg/g) was observed in pelvic muscle of jingle babbler .Whereas lowest concentration of Cu was estimated in the blood of jungle babbler

According to present study, Pb concentration in organs is statistically significant ($P<0.05$) whereas in species it is not significant ($P= 0.278$).The maximum accumulation of Pb was reported in Bank Myna (1.13µg/g)**.** Whereas highest accumulation of Cu was reported in Grey Heron which may be due to high levels in their habitat. In liver (1.06µg/g)of Grey Heron Cu accumulation was highest which is according to study of (Horai et al., 2007).In present study all livers of four species (maximum concentration 1.98 µg/g in liver of Cattle Egret) which is within range of background levels. The concentration of Pb in liver (6 µg/g) is good monitor of abnormal exposure during life of a specie and normal biological functioning (Clark & Scheuhammer, 2003). Concentration above 15 µg/g caused death due to lead poisoning(Wayland et al., 1999).Levels of Pb (6-20 µg/g) indicate exposure greater than background and signs of poisoning can be seen in birds. Higher concentration of Pb is related to acute exposure. This acute exposure can cause danger to life. If exposure is 420 µg/g, this is due to shot digestion of Pb. In this case, specie is subjected to source of lead (Pain et al., 1993).In present study, Pb concentration in liver of Grey Heron, Cattle Egret Bank Myna and Jungle Babbler is within the range of background level as (Pb < 6 µg/g) ((Hulse et al., 1980 ; Cheney et al., 1981 ; Custer & Mulhern., 1983 ; Blus et al., 1985 ; Honda et al., 1986 ; Lee et al., 1987 ; Cosson et al., 1988 ; Husain & Kaphalia, 1990 ; Hontelez et al., 1992 ; Rodgers, 1997; Kim et al., 2000; Custer et al., 2007; Kim & Koo, 2007).Pb concentration was different in organs and blood of different bird species. Dauwe et al., (2003) described that in species of House Sparrow (*Passer domesticus*), Pb was high in liver and low in muscle. Which in present study is true for Grey Heron, Cattle Egret and Jungle Babbler. Literature about toxicity of muscle tissue is very less as compared to other organs and tissues. Though we can made some comments based on accessible literature (Gasparik et al., 2010). In muscle, highest Pb concentration (3.23µg/g) was reported in pelvic muscle of jungle babbler. Which is higher than levels of Pb as described by Gasparik et al., (2010).

The levels of Pb in blood of different species was very low. In blood, Pb (0.199 µg/g) highest concentration was reported in Cattle Egret. Pb in blood of four species is in threshold limit. The background level for blood is 0.2- < 0.5 µg/g. The level <0.5 µg/g show subclinical poisoning, at 0.5-1 µg/g clinical poisoning can be seen. Whereas at >1 µg/g in blood can cause severe clinical poisoning(Franson & Pain, 2011).In blood greater amounts of lead can cause enzyme inactivity. Some enzymes inhabit at level of <5 µg/g. Lead is deposited in liver and kidney from blood. Lead in different tissues has different retention time.(Franson & Pain, 2011).Metals concentration in bird is influenced by food quality, habitat, metal availability and environmental contamination (Gasparik et al., 2010).Lead toxicity in birds can cause weakness, leg paralysis, difficulty in flying, kidney problems, heme synthesis, nervous system diseases and mortality in severe cases (Szymczyk & Zalewski, 2003).Detection of Pb indicate that birds are exposed to lead by diet, habitat or environment. Moreover leaded gasoline although banned in different countries is still used in developing countries.The reason of the presence of lead can be vehicular emission as well as the prey of Cattle Egret. The level of Pb in organs was higher than Cd and liver of birds contained highest amount of lead as compared to muscles. This is due to detoxification of liver. In this study birds are not ingested lead shots as no adverse effects can be seen.

According to present study, Cd concentration in organs is statistically significant ($P<0.05$) whereas in species it is not significant ($P= 1.479$). It means Cd is detected in organs and blood but it is far below the threshold limit.The Cd concentration is relatively lower in liver of Cattle Egret as compared to other species. Cadmium concentration in liver and muscles is related to amount of died according to (Kim & Oh, 2015).Cd in Liver of Bank Myna is highest as compared to other species which is due to longer lifespan of bank myna. As birds having longer lifespan accumulate more Cd according to (Kim & Oh, 2015) which in present study is true for Bank Myna.In present study, Cd concentration for Bank Myna, Grey Heron, Cattle Egret and Jungle Babbler (maximum concentration is 0.17µg/g in liver of Bank Myna) is far below than hazardous exposure to environment and toxicosis level. In liver Cd concentration (3 µg/g) recommend hazardous exposure to environment (Scheuhammer, 1987) and about 40 µg/g in liver is responsible for toxicosis(Kim & Oh, 2015).Cd among all four organs and blood was highest in liver of the species studied. This represents that liver is dominant target for Cd accumulation. Dauwe et al., (2005) described that Cd was high in liver and ovary, low in muscle and brain tissue of Great Tit *(Parus Major*). Saeki et al., (2000) studied that in common Cormorants Cd was highest in liver as compared to muscle, brain and bone. Various studies showed highest concentration of Cd in liver as compared to muscle , brain and blood (Swaileh & Sansur, 2006 : Albayrak & Mor, 2011). May be this is due to that birds detoxification system of liver.According to diet and pollution of area, heavy metal accumulation in liver, muscle and kidney show chronic exposure. Because muscle is site for accumulation and deposition and liver is site of detoxification in body(Naccari et al., 2009).Scheuhammer, (1987) used liver to check Cd in order to monitor biological exposure. Because in liver cadmium is stable and present in half of body burden. The reason of high Cd levels in liver may be due to the fact that liver is resilient to adverse effects of Cd (Furness, 1996).In present study, levels of Cd in livers of all species are within background limits according to the study of (Scheuhammer, 1987; Hulse et al., 1980; Cheney et al., 1981; Custer & Mulhern, 1983; Blus et al., 1985; Cosson et al., 1988; Husain & Kaphalia, 1990; Hontelez et al., 1992; Guitart et al., 1994; Mora & Anderson, 1995; Rattner et al., 2000; Custer et al., 2007; Kim & Koo, 2007)

Toman et al., (2005) showed pectoral muscle contain less Cd levels as compared to liver and kidney.Which is true for present study except in case of Cattle Egret. The highest Cd (0.18 µg/g) concentration was reported in pelvic muscle of bank myna. Which is far below the toxic level.There are few studies present that report Cd in muscle of birds is 0.2-5% of kidney concentration present (Cheney et al., 1981).The accumulation of Cd and Fe in soft tissue (liver) is greater as compared to hard tissues (Honda, Min, & Tatsukawa, 1986).The concentration of Cd in blood is was in range of 0.082-0.121 µg/g. Cd in blood should be less than 0.5 µg/g (Franson & Pain, 2011). So, in present study Cd is not responsible for adverse effects in birds.Cadmium is extremely toxic for birds. It causes damage to lungs and digestive system. In liver and kidneys it can easily move and deposit in body. In higher amount , it disturbs the activity of different enzymes. It produces cancer in birds (Szymczyk & Zalewski, 2003).Pb and Cd are very good indicator of native environmental pollution in Grey Heron and Cattle Egret. They are mostly selected by ecotoxicologists for estimation of trace metals in the environement (Boncompagni et al., 2003).Cr in case of species was not statisticallysignificant (P= 0.135). Whereas in organs, significant difference was present (P<0.01).The highest Cr concentration (0.45 µg/g) was reported in pectoral muscle of Jungle Babbler. Higher amounts of Cr can cause adverse impacts. Concentration greater than 4 µg/g indicate Cr poisoning (Outridge & Scheuhammer, 1993).In present study chromium concentration is in threshold limit.

Kekkonen et al., (2012) reported chromium absorption of 0.23±0.03 µg/g and 0.18±0.02 µg/g, which is very low in liver of House sparrows collected from sub urban and rural areas in southern Finland. Chromium causes adverse and carcinogenic effects in large amounts. Kler et el.,(2014) testified that in mallard duck high concentration of chromium was responsible for reproductive impairment. The difference of chromium concentration in different organs may be due to nature of their different feeding habitat and nature of human activities such as use of chromium containing fertilizers. Cu in case of species (P= 0.01) and organs (P<0.01) was statistically significant. It means significant difference was present in species and organs. The highest accumulation of copper (1.891 µg/g) was reported in liver of cattle egret. For growth and development copper is a vital element. It is very essential for cell functioning. In livers of birds high copper concentration was linked with low protein and fats (Pappas et al., 2006). Mean copper concentration in the blood of cockatiels was 0.168 ± 0.222 µg/g proposed by Howard, (1992). Present study reported maximum range of 0-0.046 µg/g which is far below than the range described by Howard. The Cu in liver of egret was lower than herons from the species collected from South Korea (Honda et al., 1986). Which is true in case of present study as Cu in liver of Grey Heron is 0.77µg/g and in Cattle Egret is 1.98µg/g. The amount of Cu in each organ of birds is different due to difference in growth rate and essential requirement of element for each organ. Migration of birds can also cause difference in metal accumulation. Copper is an essential element its deficiency in birds can cause thinning of capillary barrier of lungs, reduction in bone strength and testicular atrophy (Osofsky et al., 2006).In higher amounts copper is toxic for birds. There are different sources through which birds are exposed to copper.

Leaching from copper containers and copper piping for water can cause increase amount of Cu in birds.

Comparison between different Feeding Guilds: In liver of four species, Pb and Cu were highest in invertebrate predator, Cd in omnivore and Cr in granivore. For pectoral muscle, omnivore species contained highest amount of Pb and Cd followed by Cu in carnivore and Cr in granivore. For pelvic muscle, Pb was highest in granivore followed byCu in granivore, Cd in omnivore and Cr in invertebrate predator. Highest Cd accumulation was recorded inblood of invertebrate predator. So, accumulation of trace metals were different in tissues of different feeding groups.Differences in the accumulation of trace metals are related to different feeding routines of birds. (Dauwe et al., 2003). In present study, highest mean of Pb and Cr in the four trophic levels has been reported in granivores. Whereas highest mean concentration of Cd was reported in omnivore species and Cu in invertebrate predator followed by carnivore and omnivore. So, different metals influenced different feeding groups. The results showed that omnivore species are good indicator of Cd exposure.

Comparison between terrestrial and aquatic species: Mostly in literature, importance has been given to the relationship among concentration of trace metals in bird organs with gender, age, diet, habitat and concentration of exposure (Burger & Gochfeld, 2000; Ochoa-Acuna et al., 2002; Berglund et al., 2011; Dauwe et al., 2003; Leonzio et al., 2009; Pan et al., 2008; Zolfaghari et al., 2009; Castro et al., 2011; Jerez et al., 2011; Frantz et al., 2012)

In broader way, we anticipated that terrestrial birds are more vulnerable to metal pollution as compared to aquatic birds. Though this pattern did not remain same for all organs but we can say that terrestrial species such as Bank Myna and Jungle Babbler accumulated more trace metals as compared to Cattle Egret and Grey Heron (Aquatic species). The reason may be due to their position in trophic level and degree of metal exposure. Cu in aquatic species is very high, may be due to the run off generated from soil contaminated by copper based agricultural fungicides. Highest concentration of Cu exist in aquatic species which shows greater concentration of Cu in wetlands of Punjab, Pakistan

Limitations of the study: The current study investigate the accumulation of trace metals in tissues of birds and compare difference between aquatic and terrestrial species and different feeding guilds. But this study has certain limitations. Present study is conducted on internal organs. Choice of feathers for analysis would be a better choice because they are easy to obtain and there is no need to sacrifice a bird. But feathers has certain limitations too. As results may be influenced by types of feathers and location of body parts. Avifauna is not study on basis of age and gender. Because of restricted distribution ranges of some species it was very tough to gather samples of same species from every site. As, sampling was not specifically done for this study. Birds were captured for the framework of other study. Heavy metal toxicity has to be determine for a period of a year to determine heavy metal toxicity pattern in food web.

Conclusion: The present work provides information about heavy metals (Pb, Cd, Cr and Cu) in different organs of birds collected from Manga Mandi, District Lahore, Punjab, Pakistan.Heavy metals concentration varied between four species of birds. All trace metals were within theirpermissible limit. Their concentration did not recommend any harmful effects to the birds. The present study clearly reported significant difference between the organs of birds. Whereas in case of species significant difference was only reported in Cu. Livers of four species revealed highest concentration of lead. Lowest concentration of Cd was reported in all species. Species revealed heterogeneous levels of metals in all organs. Mean concentration (µg/g) of trace metals in organs and blood of Bank Myna and Jungle Babbler (terrestrial species) followed the trend: Pb > Cu > Cr > Cd except blood of both species whereas in Cattle Egret and Grey Heron (aquatic species) the trend was following: Cu > Pb > Cr > Cd with exception of liver in Cattle Egret, pelvic muscle in Grey Heron and blood in both species. Trace metals concentration were very low in blood of species. Some samples of blood did not detect any metals. The reason of low chromium may be improvement of metabolic processes in bird species. Liver and blood samples are used most widely for determining contemporary and long term exposure of metals. There was no significant difference ($P>0.05$) between habitat and feeding guilds of birds. We anticipated that terrestrial birds are more vulnerable to metal pollution as compared to aquatic birds. Though the pattern did not remain same for all organs. Metal accumulation in Heron and egret provide information about the overall ecosystem and threats to organisms as they are at top of food chain. No strong evidence was found that suggest any reproductive or immune disorder. Although, metals are within threshold limit, this does not call for concern since permissible limit for birds is not surpassed. However, continuous monitoring of metals are necessary. The accumulation of metals in birds will help us to estimate the surroundings of ecosystem. The present study provides a reference point for forthcoming research to better understand the accumulation of metals in birds, how human activities effects avifauna and ways of how accumulation occurs.

REFERENCES

Hosseini, M., Nabavi, S. M. B., Nabavi, S. N., & Pour, N. A. (2015). Heavy metals (Cd, Co, Cu, Ni, Pb, Fe, and Hg) content in four fish commonly consumed in Iran: risk assessment for the consumers. Environmental monitoring and assessment, 187(5), 237.

Swaileh, K. M., & Sansur, R. (2006). Monitoring urban heavy metal pollution using the House Sparrow (Passer domesticus). *Journal of Environmental Monitoring*, *8*(1), 209–213. https://doi.org/10.1039/b510635d

Burger, J., Gochfeld, M., Sullivan, K., & Irons, D. (2007). Mercury, arsenic, cadmium, chromium lead, and selenium in feathers of pigeon guillemots (Cepphus columba) from Prince William Sound and the Aleutian Islands of Alaska. *Science of the Total Environment*, *387*(1–3), 175–184. https://doi.org/10.1016/j.scitotenv.2007.07.049

Malik, R. N., Chaudhry, M. J. I., Abbasi, N. A., Jaspers, V. L. B., & Ali, S. (2014). Influence of taxa, trophic level, and location on bioaccumulation of toxic metals in bird's feathers: A preliminary biomonitoring study using multiple bird species from Pakistan. *Chemosphere*, *120*, 527–537. https://doi.org/10.1016/j.chemosphere.2014.08.054

Movalli, P.A. (2000). Heavy metal and other residues in feathers of laggar falcon Falco biarmicus jugger from six district of Pakistan. *Environmental Pollution*, 109, 267–275.

Naccari, C., Cristani, M., Cimino, F., Arcoraci, T., & Trombetta, D. (2009). Common buzzards (Buteo buteo) bio-indicators of heavy metals pollution in Sicily (Italy). *Environment International*, *35*(3), 594–598. https://doi.org/10.1016/j.envint.2008.11.002.

Battaglia, A., Ghidini, S., Campanini, G., & Spaggiari, R. (2005). Heavy metal contamination in little owl (Athene noctua) and common buzzard (*Buteo buteo*) from northern Italy, *60*, 61–66. https://doi.org/10.1016/j.ecoenv.2003.12.019

Szymczyk, K., & Zalewski, K. (2003). Copper, zinc, lead and cadmium content in liver and muscles of mallards (Anas platyrhychnos) and other hunting fowl species in Warmia and Mazury in 1999-2000. *Polish Journal of Environmental Studies*, *12*(3), 381–386.

Naccari, C., Cristani, M., Cimino, F., Arcoraci, T., & Trombetta, D. (2009). Common buzzards (Buteo buteo) bio-indicators of heavy metals pollution in Sicily (Italy). *Environment International*, *35*(3), 594–598. https://doi.org/10.1016/j.envint.2008.11.002.

Roux, K. E., & Marra, P. P. (2007). The presence and impact of environmental lead in passerine birds along an urban to rural land use gradient. *Archives of Environmental Contamination and Toxicology*, *53*(2), 261–268. https://doi.org/10.1007/s00244-006-0174-4

Burger, J. (1993).Metals in avian feathers: bio indicators of environmental pollution. .*Environmental Toxicology*. 5, 203–311.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R., & Eens, M. (2000). Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? *Archives of Environmental Contamination and Toxicology*, *39*(4), 541–546. https://doi.org/10.1007/s002440010138

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R., & Eens, M. (1999). Are eggshells and egg contents of Great and Blue Tits suitable as indicators of heavy metal pollution? *Belgian Journal of Zoology*, *129*(2), 439–447.

Jarosław, S., & Ban, M. (2013). Avian Feathers as Bioindicators of the Exposure to Heavy Metal Contamination of Food, 302–305. https://doi.org/10.1007/s00128-013-1065-9

Dauwe, T., Chu, S. G., Covaci, A., Schepens, P., & Eens, M. (2003). Great Tit (Parus major) Nestlings as Biomonitors of Organochlorine Pollution. *Archives of Environmental Contamination and Toxicology*, *44*(1), 89–96. doi:10.1007/s00244-002-1243-y

Tsipoura, N., Burger, J., Feltes, R., Yacabucci, J., Mizrahi, D., Jeitner, C., & Gochfeld, M. (2008). Metal concentrations in three species of passerine birds breeding in the Hackensack Meadowlands of New Jersey. *Environ. Res.* 107, 218–228.

Van Straalen, N.M., & Ernst, W. H. O. (1991). Metal biomagnification may endanger species in critical pathways. *Oikos*, 62(2): 255-256. doi :10.2307/3545272

Boncompagni, E., Muhammad, A., Jabeen, R., Orvini, E., Gandini, C., Sanpera, C., Fasola, M. (2003). Environmental C contamination a n d T Egrets as Monitors of Trace-Metal Contamination in Wetlands of Pakistan, *406*, 399–406. https://doi.org/10.1007/s00244-003-0198-y

Swaileh, K. M., & Sansur, R. (2006). Monitoring urban heavy metal pollution using the House Sparrow (Passer domesticus). *Journal of Environmental Monitoring*, *8*(1),209213.https://doi.org/10.1039/b510635d.

Burger, J., & Gochfeld, M. (1998). Metals in albatross feathers from Midway Atoll: influences of species, age, and nest location. *Environmental Research*, 82, 207–221.

Janssens, E., Dauwe, T., Pinxten, R., Bervoets, L., Blust, R., & Eens, M. (2003). Effects of heavy metal exposure on the condition and health of nestlings of the great tit (Parus major), a small songbird species. *Environmental Pollution*, *126*(2), 267–274.

Vashishat, N., & Kler, T. K. (2014). Birds As Bioindicators of Heavy Metal, 1–6.

Szymczyk, K., & Zalewski, K. (2003). Copper, zinc, lead and cadmium content in liver and muscles of mallards (Anas platyrhychnos) and other hunting fowl species in Warmia and Mazury in 1999-2000. *Polish Journal of Environmental Studies*, *12*(3), 381–386.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R., & Eens, M. (2000). Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? *Archives of Environmental Contamination and Toxicology*, *39*(4), 541–546. https://doi.org/10.1007/s002440010138

Berglund, Å. M. M., Koivula, M. J., & Eeva, T. (2011). Species- and age-related variation in metal exposure and accumulation of two passerine bird species. *Environmental Pollution*, *159*(10), 2368–2374. https://doi.org/10.1016/j.envpol.2011.07.001

Horai, S., Watanabe, I., Takada, H., Iwamizu, Y., Hayashi, T., Tanabe, S., & Kuno, K. (2007). Trace element accumulations in 13 avian species collected from the Kanto area, Japan. *Science of the Total Environment*, *373*(2–3), 512–525. https://doi.org/10.1016/j.scitotenv.2006.10.010

Burger, J., & Gochfeldf, M. (2000). Metals in albatross feathers from midway atoll: Influence of species, age, and nest location. *Environmental Research*, *82*(3), 207–221. https://doi.org/10.1006/enrs.1999.4015

Ochoa-Acuna, H., Sepúlveda, M., Gross, T. (2002). Mercury in feathers from Chilean birds: influence of location, feeding strategy, and taxonomic affiliation. *Mar. Pollut. Bull*. 44, 340–345.

Berglund, Å. M. M., Koivula, M. J., & Eeva, T. (2011). Species- and age-related variation in metal exposure and accumulation of two passerine bird species. *Environmental Pollution*, *159*(10), 2368–2374. https://doi.org/10.1016/j.envpol.2011.07.001

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R., & Eens, M. (2003). Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environmental Pollution*. 124, 429–436.

Leonzio, C., Bianchi, N., Gustin, M., Sorace, A., Ancora, S. (2009). Mercury, lead and copper in feathers and excreta of small passerine species in relation to foraging guilds and age of feathers. *Bull. Environ. Contam. Toxicol*. 83, 693–697.

Pan, C., Zheng, G., Zhang, Y. (2008). Concentrations of metals in liver, muscle and feathers of tree sparrow: age, inter-clutch variability, gender, and species dif- ferences. *Bull. Environ. Contam. Toxicol.* 81, 558–560.

Zolfaghari, G., Esmaili-Sari, A., Ghasempouri, S.M., Baydokhti, R.R., Hassanzade Kiabi, B. (2009). A multispecies-monitoring study about bioaccumulation of mercury in Iranian birds (Khuzestan to Persian Gulf): effect of taxonomic af- filiation and trophic level. *Environ. Res*. 109, 830–836.

Castro, I., Aboal, J.R., Fernandez, J.A., Carballeira, A. (2011). Use of raptors for bio- monitoring of heavy metals: gender, age and tissue selection. *Bull. Environ. Contam. Toxicol*. 86, 347–351.

Jerez, S., Motas, M., Palacios, M.J., Valera, F., Cuervo, J.J., Barbosa, A. (2011). Con- centration of trace elements in feathers of three Antarctic penguins: geo- graphical and interspecific differences. *Environ. Pollut*. 159, 2412–2419.

Frantz, A., Pottier, M.A., Karimi, B., Corbel, H., Aubry, E., Haussy, C., Gasparini, J., Castrec-Rouelle, M. (2012). Contrasting levels of heavy metals in the feathers of urban pigeons from close habitats suggest limited movements at a restricted scale. *Environ. Pollut*. 168, 23–28.

Horai, S., Watanabe, I., Takada, H., Iwamizu, Y., Hayashi, T., Tanabe, S., & Kuno, K. (2007). Trace element accumulations in 13 avian species collected from the Kanto area, Japan. *Science of the Total Environment*, *373*(2–3), 512–525. https://doi.org/10.1016/j.scitotenv.2006.10.010

Clark, A.J., & Scheuhammer, A.M. (2003). Lead poisoning in up-land- foraging birds of prey in Canada. *Ecotoxicology*,12, 23–30.

Wayland, M., Neugebauer, E., & Bollinger, T. (1999). Concentrations of lead in liver, kidney, and bone of bald and golden eagles. *Archives of Environmental Contamination and Toxicology*, *37*(2), 267–272. https://doi.org/10.1007/s002449900514

Pain, D. J., & C. Amiard-Triquet. (1993). Lead poisoning in raptors in France and elsewhere. *Ecotoxicology and Environmental Safety*, 25,183–192.

Hulse, M., Mahoney, J. S., Schroder, G. D., Hacker, C. S., & Pier, S. M. (1980). Environmentally acquired lead, cadmium, and manganese in the cattle egret, Bubulcus ibis, and the laughing gull, Larus atricilla. *Archives of Environmental Contamination and Toxicology*, *9*(1), 65–77. https://doi.org/10.1007/BF01055500

Cheney, M. A., Hacker, C. S., & Schroder, G. D. (1981). Bioaccumulation of lead and cadmium in the Louisiana heron (Hydranassa tricolor) and the cattle egret (Bubulcus ibis). *Ecotoxicology and Environmental Safety*, *5*(2), 211–224. https://doi.org/10.1016/0147-6513(81)90036-1

Custer, T. W., & Mulhern, B. L. (1983). Heavy metal residues in prefledgling black-crowned night-herons from three Atlantic coast colonies. *Bulletin of Environmental Contamination and Toxicology*, *30*(1), 178–185.doi:10.1007/bf01610118

Blus, L. J., Henny, C. J., Anderson, A., & Fitzner, R. E. (1985). Reproduction, Mortality, and Heavy Metal Concentrations in Great Blue Herons from Three Colonies in Washington and Idaho. *Colonial Waterbirds*, *8*(2),110.doi:10.2307/1521060

Honda, K., Min, B.Y., Tatsukawa, R. (1986). Organ and tissue distribution of heavy metals, and age-related changes in the eastern great white egret Egretta alba modesta in the Korea. *Arch Environ Contam Toxicol* , *15*,185–197.

Lee, P., Honda, K., Tatsukawa, R. (1987). Comparison of tissue distribution of heavy metals in birds in Japan and Korea. *J Yamashina Inst Ornithol*, *19*,103–116.