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ESTIMATION OF METAL CONTENT IN AVIAN FAUNA OF SIALKOT, PAKISTAN USING FEATHERS AS BIO-INDICATOR

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ABSTRACT

The intent of the present study was to determine the concentrations of metal content in feather samples of different avian species collected from different areas of Sialkot, Pakistan. For this purpose atomic absorption spectrophotometer was used to estimation the metals concentration. The estimated concentration (μ g/g) of these metals were 104.89±22.39, 23.19±1.23, 15.68±3.50, 10.18±2.47, 5.38±1.50, 1.52±0.02 for Zinc> lead> chromium> copper> nickel> cadmium respectively. The concentration of cadmium was found to be lower than the threshold level. However, continuous exposer to metals may result in the bio-magnification of metals in body especially in soft tissues such as liver, kidneys which is harmful for avian fauna. The concentrations of lead and chromium in the feather samples were found to be more than the approved /required levels of these minerals may be the one reason for the decline of avian fauna in study area. The main sources of metal pollution are the tanneries and surgical industries in study area as these industries use lead, chromium and zinc salts extensively. Most of these industries dispose of their untreated waste water in the naturel water resources such as in river and canals threatening to the aquatic flora and fauna. Results of the present study revealed that feathers may be used as bio-indicator for estimation of environmental pollution. The findings will also highlight the threats to the ecology and diversified avian fauna of the study area.

Keywords; Avian fauna, Bio-indicator, Environmental pollution, Feathers, Metal contents.

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INTRODUCTION

Metals having density greater than 5 g/cm³ and atomic number greater than 20 are known as heavy metals. Metals and metalloids contaminations may have harmful impacts on ecology and living organisms of that ecosystem. Metals are classified according to their density, relative atomic mass and atomic number (Ali and Khan 2018). As these heavy metals [Mercury (Hg), Chromium (Cr), Cobalt (Co), Copper (Cu), Selenium (Se), Zinc (Zn), Arsenic (As), Manganese (Mn)] can enter in to the food chain, thus, a continuous accumulation of these metals may cause biomagnification. These heavy metals are reported to reduce the reproductive performance in birds (Abdullah et al. 2015). Although these metals are required in trace amount for the normal physiological functions and development, however a concentrations more than threshold level is toxic to fauna and flora (Einoder et al. 2018).

Due to their non-biodegradable nature and long half-lives these metals are serious threats to living organisms and have been led to the extinction of various

wild species (Ullah et al. 2014). Findings reveal that heavy metals may accumulate in various body parts of the organism, particularly in the hairs, bones, eggs, liver, blood, kidney and plumes (Einoder et al. 2018). Metals are major anthropogenic source of pollution in industrial areas which routinely affect our food web (Einoder et al. 2018). The bioaccumulation of metals occurs if the uptake of metals is increased (Eagles-Smith et al. 2016; Altaf et al. 2014). Raised concentration of metals has caused many health related problems in birds e.g. deterioration of eggshell quality, reduction in reproductive performance, and immunosuppression thus rendering a serious threat to avian population. Morphometric parameters, for example, the weight; bone structure and wing length are directly affected by the accumulation of metals in the body of bird (Sharma and Vashishat 2017).

Just only the analysis of metallic contaminants in the soil, air and water do not provide sufficient information about the harmful aspects of these metals on the living organisms because the avian species feed on a wide range of varying diets. Thus estimation of metals level in birds may give better information about the

ecological risks to the flora and fauna of the study area (Zarrintab et al. 2016). Avian fauna is best to be chosen as an ideal bio-indicators of an area because the ideal bioindicators must be widely distributed and easy to capture, territory size should be well defined, individual size should be large if the organs are used for assessments, the biology of individual should be well known and the breading of species should be possible in captivity (Aazami and KianiMehr 2018). The delicate tissues (liver, kidney, spleen, heart, and lung etc.) are comprehensively used to investigate the accumulation of various metals from the ecosystem. Further, the accumulation of a metal by different organs of the same organism varies from organ to organ so it is necessary to estimate the level of metals in the different organs of the organism for the better evaluation (Wei and Ma 2011).

Feathers receive the metals from the circulatory system or from the food sources that they currently used as feed during the development of plumes. Feathers are associated with the circulatory system through the veins, and the metals can bind with molecules of protein in the feathers (Manjula *et al.* 2015). Avian quills can be used as bio-indicators for the assessment of metal contamination level. The level of these metals is greater in plumes (avian species discharge significant measures of metals through plume shed) than in other tissues, and consequently simpler to collect and evaluate for the estimation of metals contamination level (Abdullah *et al.* 2015).

Like other developing countries Pakistan is also development and facing the issue under industrialization, urbanization, and over-population (Qureshi et al. 2015). There are various threats to the diversity of avian species among these; increase in cultivation of land; illegal hunting, loss of natural habitat and aquatic pollution (Altaf et al. 2013). Industrial wastewater hold many poisonous metals continuously enter in the water ecosystem posing threats to life (Qadir and Malik 2009; Hashmi et al. 2013) and these metals may be carcinogenic, mutagenic and teratogen (Allen 1994; Alloway 2013).

Sialkot is known worldwide for its sports products, surgical and tanning industry. The tanning industry produces waste water containing more than 17 different types of lethal chemicals and metals used in leather industry (Iqbal *et al.* 1998). According to Mahmood and Malik (2014), Sialkot generated nineteen million cubic meter waste water every year and the production of waste water is continuously increasing due to the establishment of heavy industry in the area. Most of the industries have no waste water treatment system, so the contaminated water is thrown untreated in natural water bodies posing serious life threats to aquaculture and avian species. In the present study, the levels of (Cr, Ni,

Cu, Zn, Cd and Pb) were investigated in various bird species of Sialkot by using bird's feathers as bioindicator. Results of the current study will be helpful for management, protection and improvement of avian fauna and environment of the study area.

MATERIALS AND METHODS

Study Area: Feather samples from birds were collected from different sites of Sialkot, Pakistan as shown in Fig1. The GPS coordinates of sampling sites are given in Table 1.

Sampling: Birds were captured by using mist net with mesh size of 30-38 mm (Gushit *et al.* 2016). Mist net was placed randomly to capture the birds in the morning at varying time from 5am- 11am. Mist net was checked after every 15-20 minutes to prevent the captured bird from stress. Feather samples of 48 birds (*n*=48) belong to 18 different species were collected from different areas of Sialkot (see Table 1 and Figure 1). The captured birds were immediately released after taking feather samples. The feather samples were collected in plastic bags and shifted to the lab of the Department of Wildlife and Ecology, University of Veterinary and Animal Sciences Lahore-Pakistan and stored at -20 C in refrigerator as described by Abdullah *et al.* (2015).

Metal analysis: Metal (Cr, Ni, Cu, Zn, Cd and Pb) concentration was analyzed in the feather samples. The feather samples were washed with tap water first then cleaned with distilled water and 1 M acetone (3 times) for the removal of any external contaminants. Samples were air dried and then in an oven for 2-3 hours at 105 °C. Then feathers were cut into small pieces by using stainless steel scissor to allow easy digestion. 0.5g of feather sample was transferred into quartz crucibles and 0.25 ml of HClO₄ and 1.0 ml of HNO₃ were added into the crucible, which were covered with lid. The digestion of samples were performed by using a hot plate, initially at low temperature and then on high temperature. The digestion of samples was continuing until the mixture become pale yellow. Completely digested samples were filtered and diluted with 50ml of distilled water and stored in sample bottles (Nighat et al. 2013). The analyses of metal contents in digested samples were performed as described by Gushit et al., (2016).

Statistical Analysis: Descriptive statistical analysis was used for the analysis of data. The data were analyzed by one way analysis of variance (ANOVA) using the SAS (SAS 9.3 2011). A post hoc Tucky test was used to compare differences among individual means. P < 0.05 was considered statistically significant.

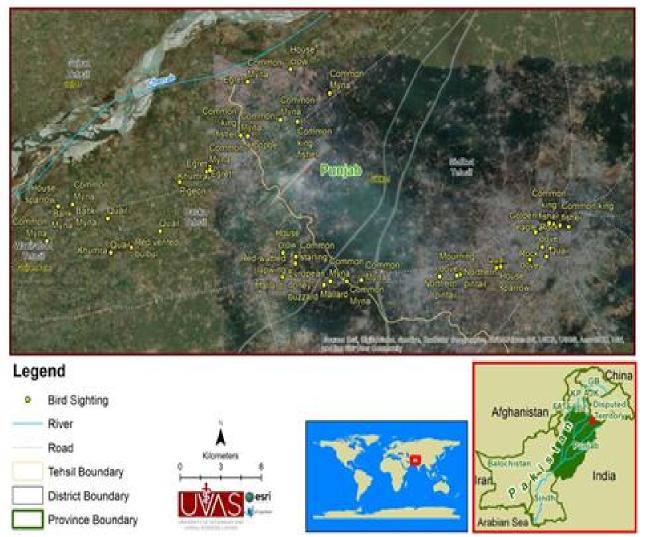


Fig 1. Sites from where feather samples were collected for estimation of metal contents (study area).

Table 1. Table shows the sampling of birds from various areas of Sialkot and their respective GPS location.

No.	Common name	Scientific name	Sample quantity	Longitude	Latitude
				E074 ^o 18.458	N32 ^o 32.523
				E074 ^o 21.263	N32 ^o 34.123
				E074 ^o 23.221	N32 ^o 35.017
				E074 ^o 19.952	N32 ^o 35.394
1	C M	1 1 - 41 4 4	10	E074 ^o 19.676	N32 ^o 33.592
1	Common Myna	Acridotheres tristis	10	E074 ^o 11.960	N32 ^o 30.045
				E074 ^o 12.971	N32 ^o 31.284
				E074°23.242'	N32°28.697'
				E074°23.908'	N32°28.692'
				E074°24.485'	N32°28.731'
				E074 ^o 19.676	N32 ^o 33.592
2	C 1-i £1	411441	4	E074 ^o 21.938	N32 ^o 34.065
2	Common king fisher	Alcedo atthis	4	E074°32.395'	N32°30.577'
				E074°32.153'	N32°30.611'
2	F 4	4 1 11	2	E074 ^o 19.952	N32 ^o 35.394
3	Egret	Ardea alba	3	E074 ^o 18.337	N32 ^o 32.392

-				E074 ^o 18.452	N32 ^o 32.430
				E074 ^o 14.505	N32 ^o 29.652
				E074 ^o 16.466	N32 ^o 30.376
4	Quail	Coturnix	5	E074 ^o 14.387	N32 ^o 30.804
				E074°31.853'	N32°29.528'
				E074°29.852'	N32°29.146'
5	Bank Myna	Acridotheres ginginianus	2	E074 ^o 12.971	N32 ^o 31.284
3	Dank Wiyna	Acridomeres gingimanus	2	E074 ^o 12.971	N32 ^o 31.284
6	House sparrow	Passer domesticus	2	E074 ^o 12.415	N32 ^o 31.218
	-			E074°30.025'	N32°29.188'
7	Red vented bulbul	Pycnonotus cafer	1	E074 ^o 15.315	N32 ^o 29.834
8	Pigeon	Columba livia	1	E074 ^o 17.243	N32 ^o 32.038
9	House crow	Corvus splendens	2	E074 ^o 21.653	N32 ^o 35.828
		•		E074°21.358'	N32°29.653'
10	Hoopoe	Upupa	1	E074 ^o 19.956	N32 ^o 33.575
11	Khumra		2	E074 ^o 18.450	N32 ^o 32.428
				E074 ^o 14.505	N32 ^o 29.652
12	Red-wattled lapwing	Vaneius indicus	1	E074°21.628'	N32°29.053'
13	Mourning dove	Zenaida macroura	2	E074°27.595'	N32°28.854'
_	8			E074°31.187'	N32°29.418'
14	European honey buzzard	Pernis apivou	2	E074°32.015'	N32°29.799'
	1	1		E074°21.861'	N32°29.290'
15	Common starling	Strunus vulgaris	2	E074°32.744'	N32°30.521'
	٥	0		E074°21.880'	N32°29.524'
16	Golden eagle	Aquilla Chrysaetos	2	E074°31.964'	N32°30.650'
	2	1		E074°31.622'	N32°30.450'
17	Rock dove	Columba livia	2	E074°31.415'	N32°30.340'
				E074°31.686'	N32°29.848'
18	Northern pintail	13.Anas acuta	2	E074°28.409'	N32°28.915'
	•			E074°28.280'	N32°28.878'
19	Mallard	Anas platyrhynchos	2	E074°22.995'	N32°28.570'
		1 , ,		E074°21.333'	N32°28.845'

RESULTS AND DISCUSSIONS

The findings of the current study about the concentrations of various metal contents in avian feathers are presented in Table 2. The results of the present study revealed that concentration of heavy metals in feathers samples of various species of birds were found in the following order, Zn> Pb> Cr> Cu> Ni> Cd measuring the 104.89 ± 22.39 , concentration 23.19 ± 1.23 . $(\mu g/g)$ 15.68 ± 3.50 , 10.18 ± 2.47 , 5.38 ± 1.50 and 1.52 ± 0.02 respectively. These findings are comparable with the concentrarions of metal contents in avian feathers reported by various researchers from different parts of world (Table 3) and from various parts of Punjab, Pakistan (Table 4 and Figure 2).

Nickel: The concentration of nickel (Ni) reported in the avian feathers samples during this study was 5.38 ± 1.50 µg/g. There are evidences that a high level of accumulated Ni in birds is toxic. According to Outridge and Scheuhammer (1993), if newly hatched chicken is fed with diet containing Ni over $300\mu g/g$, may lead to reduction in growth and death of the chicks. The birds

present in uncontaminated environment usually contain nickel in range of 0.1-5µg/g dry body weight while the birds inhabiting in polluted environment may accumulate nickel from 0.5-80µg/g dry weight. Further the high intake of nickel may lead to respiratory disorders, birth defects, and denaturing of DNA etc. (Van Wyk et al. 2001). The continuous exposure of nickel is also the one reason of many allergic reactions to human (Wilbur et al. 2008). The industries of oil and ghee are the major source of Ni in Sialkot (Qadir et al. 2008). The results of the present study revealed that the concentration of nickel in the feather samples were below than the reported level of Abdullah et al. (2015) from Lahore (41.7 µg/g) and Nighat et al. (2013) from Southern Punjab (153.4 µg/g), Central Punjab (90.3 µg/g) and Northern Punjab (42.4 ug/g). The present study reported a slightly high concentration of nickel in avian feather samples in comparison to those reported by Malik and Zeb (2009) from Chenab river (9 µg/g), Ravi river (8.1 µg/g) and Raval lake (7.8 µg/g) while greater than reported by Ullah et al. (2014) from Trimum headworks (0.1 µg/g), Shorkot (0.2 µg/g) and Mailsi (0.2 µg/g) in avian feathers.

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Sr.No	Metals	Conc. (mean ± SE)
1	Nickel (Ni)	5.38±1.50
2	Lead (Pb)	23.19 ± 1.23
3	Zinc (Zn)	104.89 ± 22.39
4	Cadmium (Cd)	1.52 ± 0.02
5	Chromium (Cr)	15.68 ± 3.50
6	Copper (Cu)	10.18 ± 2.47

Table 3. Table shows the concentration of various metal contents ($\mu g/g$) in avian feathers from present and previous international studies, a comparison.

Author	Study area	Ni	Pb	Zn	Cd	Cr	Cu
(Barbieri et al. 2010)	Pirajubaí, Santa Catarina	5.9	7.536	68.54	0.007	4.665	13.30
(Costa et al. 2011)	National Pine Forest of Quiaios)	0.78	2.35	109.78	0.12	-	11.00
	National Pine Forest of Urso)	2.15	4.89	140.10	0.38	-	10.43
(Mansouri et al. 2012)	Khuran, Iran	-	5.48	57.44	1.37	-	10.47
(Costa et al., 2013)	Figueira da Foz (Portµgal) MQ	2.38	1.27	112.9	0.03	-	8.29
	Figueira da Foz (Portµgal)MU	2.24	1.29	111.0	0.03	-	9.11
(Lester and van Riper	Arizona's upper Santa Cruz River	-	-	201.97	0.728	-	11.619
2014)	(NIWWTP)						
	Arizona's upper Santa Cruz River (TUMA)	-	-	165.87	0.545	-	10.292
(Manjula <i>et al</i> , 2015)	Tiruchirappalli, India	6.11	-	71.60	1.70	55.48	86.9
(Zarrintab et al. 2016)	Aran-O-Bidgol City, Central Iran	-	9.26	167.16	1.583	-	26.74

Table 4. Table shows the concentration of various metal contents (µg/g) in avian feathers from present and previous studies from Pakistan.

Author	Study area	Ni	Pb	Zn	Cd	Cr	Cu
(Malik and Zeb 2009)	Chenab river	9	37.5	133.8	3.1	6.6	4
	Ravi river	8.1	76.5	155.8	2.4	7.1	3.7
	Rawal lake	7.8	60.2	138.2	2.7	5.4	4
(Nighat et al. 2013)	Southern	153.4	19.5	122.2	1.8	-	16.9
	Central	90.3	21	127.2	3.4	-	21.5
	Northern	42.4	18.6	158.5	1.1	-	16.8
(Ullah et al. 2014)	Trimum Headwork	0.1	30	18.5	3.3	26.2	0.9
	Shorkot	0.2	32.5	18.8	3	30.8	0.2
	Mailsi	0.2	43.1	10.7	1.7	35.8	0.2
(Abdullah et al. 2015)	Lahore	41.7	284	529.9	41.1	21.1	52.8
Present study	Sialkot	5.38	23.19	104.89	1.52	15.68	10.18

Zinc: zinc is an essential element that plays a significant role in different catabolic and anabolic processes. Zinc is obtained from both natural and anthropogenic sources and its concentration in avian fauna depends on the availability in the ecological or biological system (Morais *et al.* 2012; Ullah *et al.* 2014). According to the study of Abdullah *et al.* (2015) there are no harmfull effects for the accumulation of zinc in high concentration and in the current study the concentration of zinc reported in avian feathers was $104.89\pm22.39 \,\mu\text{g/g}$.

The concentration of zinc in avian feathers reported in the current study are higher than the concentration of Zn reported by Ullah *et al.* (2014) in

feather samples of birds from Trimum headwork (18.5 μ g/g), Shorkot (18.8 μ g/g) and Mailsi (10.7 μ g/g). The findings of current study revealed that the concentration of Zn in avian feathers samples are lower than the findings of Abdullah *et al.* (2015), Nighat *et al.* (2013) and Malik and Zeb (2009) as showen in Table 4. The results are also comparable with the findingd of several international researches in Table 3. According to Malik *et al.* (2010) different zinc salts such as zinc sulfate, zinc acetate and zinc chloride are used in leather industry are the major source of Zn contamination.

Lead: Lead is heaviest but non-radioactive element. The major sources of lead contamination are industries and

vehicles. The industrial sewage containing salts of lead are drained in to natural water resources (Qadir and Malik 2011). In birds, the skeleton is the major storage site of lead and chromium. The abnormalities of reproductive system and body weight loss may occur in birds if exposed to high concentration of lead. Age and gender are associated with the accumulation of lead (Szefer and Falandysz 1986). Markowski *et al.* (2013) concluded that the accumulation of lead in female bones is higher than male bones, however the reason is unknown and needs to be investigated. According to the Trust *et al.* (1990) the number of white blood cells and immunity decrease in high lead-dosed Mallard ducks.

The current study reported the concentration of lead in feathers is $23.19\pm1.23~\mu g/g$ that is greater than threshold level $(4\mu g/g)$ possibly due to the heavy lead pollution from the study area (Gochfeld 2000). The current reported lead concentration in the avian feathers is below than the reported lead values of various scientists from Pakistan like Abdullah *et al.* (2015), Malik and Zeb (2009) and Ullah *et al.* (2014) as showen in Table 4. On the other hand the reported lead concentration is greater than the findings of Barbieri *et al.* (2010); Costa *et al.* (2011); Mansouri *et al.* (2012); Costa *et al.* (2013); Zarrintab *et al.* (2016) from various parts of the world (Table 3).

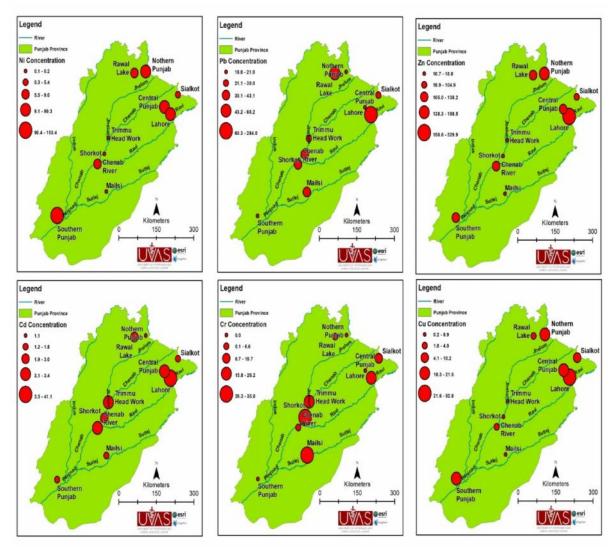


Fig 2. Figure shows a comparative analysis of concentration of various metal contents (μg/g) in different avian species from previous and present study.

Cadmium: Cadmium and its salts are more soluble making it more available to the living organisms. Once the cadmium contaminants enter in the body of

organisms, it remains here for a very long period of time (half-life of 38 years). The cadmium contamination occurs through the use of various fertilizers and

pesticides on crops (Qadir et al. 2000). Such crops are the major source of dissemination of Cd in living organisms. The coal combustion, iron and steel production, various industries (Cd batteries and paints etc.) and various pesticides are the major sources of Cd in air (Komarnicki 2005). It may affect the metabolic process by the replacement of essential elements so the exposure to cadmium may result the deficiency of essential elements (Abdullah et al. 2015). The concentration of cadmium in avian species in study area was 1.52±0.02 µg/g. The threshold limit for cadmium is considered as 2µg/g according to Gochfeld (2000). The reported cadmium concentrations in feather are lower than the values reported by Abdullah et al. (2015) from lahore (41.1 $\mu g/g$), Nighat et al. (2013) from central punjab (3.4 $\mu g/g$) and Ullah et al. (2014) from Trimium headworks and Mailsi $(3.3 \mu g/g)$.

Chromium: Fertilizers, utensils, cigarette, cosmetics and jewelry are the major sources of chromium. Lungs, kidneys and hormones producing tissues are the target sites for accumulation of chromium (Malik and Zeb, 2009). Long term exposure of Chromium may result in reduction of body weight, liver damages, heart strokes, skin disease, cancer, headache and respiratory disorders are caused by the long term exposure to chromium (Gochfeld, 2000). The concentration of chromium reported in avian feathers is 15.68±3.50 µg/g which is greater than the findings of Malik and Zeb (2009) reported in the birds feathers from Chenab river (6.6 $\mu g/g$), Ravi river (7.1 $\mu g/g$) and Rawal lake (5.4 50 $\mu g/g$). The major source of chromium contamination in the study area is use of chromium salts in leather industry and drainage of untreated water from these industries in the form of sewage and sludge (Qadir et al. 2008).

Copper: Copper is an essential element for some metabolic activities because it is the part of several enzymes such as tyrosinase, uricase, and cytochrome oxidase, which are mainly involved in oxidation reaction reactions (Turnlund, 1998), however long term exposer cause adverse effects on reproductive, respiratory and gastrointestinal system (Chen et al. 1993). The concentration of copper reported in the current study is 10.18±2.47 μg/g which is higher than the findings of Malik and Zeb (2009) and Ullah et al. (2014) but lower than the reported values by Abdullah et al. (2015) and Nighat et al. (2013) as showen in Table 4. Hematological. hepatic, endocrine and ocular defects may occur due to accumulation of copper in various body organs such as liver, brain, heart kidneys and skeletal muscles (Chen et al. 1993).

Conclusions: The feathers can be used as bio-indicators to assess the metal contamination level. Further, feathers are easy and safe to collect. The findings of the present study concluded that the metal contents reported in avian

feathers are not at safe level which is a serious threats to the biodiversity as well as human being.

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