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Hematological status of avian species along a metal pollution gradient at Sialkot, Pakistan

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Abstract: The concentration of metals and effects of these metals on hematological parameters were examined in avian species by using feathers and blood samples collected from different sites of Sialkot. The major reason for significant differences in hematological parameters and concentrations of metal contents between different species was the difference in feeding habitat. Metal concentrations in feathers and blood samples indicated that the chromium and lead were not found at a safe level. The results did not revealed a significant negative association between metal contents and hematological parameters but concluded that increased metal contents exert negative effects on hematological status especially lead and chromium, while white blood cells showed a significant positive correlation with increased metal contents except cadmium. The feathers accumulate highest concentration of metals than blood, the most probable reason behind that is the feathers reflect exogenous contamination and blood only represents the immediate dietary sources of contamination. It concluded that the feathers of avian species can be used as an efficient bio-indicator to assess the metal contamination level in an environment, and the assessment of hematological parameters can be used to find out the negative impact of metal contamination on health status. The findings of this study will help to manage environmental pollution and its impacts on wildlife.

Key words: Environmental pollution, feathers, heavy metals, hematological parameters, sialkot

1. Introduction

Environmental degradation takes place due to the increasing human population and the development of technology (Ayyamperumal et al., 2006; Qadir and Malik, 2011; Xu et al., 2013). The environment is contaminated with the passage of time by different types of contaminants, out of which metals are important pollutants because metals are non-biodegradable. Both natural and anthropogenic activities are sources of metal contamination. The metals are persistent, so these metals accumulate in the body and the level of these metals increases at each trophic level. The presence of metallic contaminants is the major threat to the natural ecosystem (Deng et al., 2007; Naccari et al., 2009). Various previous studies revealed that the accumulation of metallic contaminants causes many adverse effects such as disorders of endocrine and nervous system, mutations in genes, and abnormalities in physiology and behavior (Burger and Gochfeld, 2000; Dauwe et al., 2004; Martin et al., 2003). The number of red blood cells, amount of hemoglobin, and haematocrit represents the oxygen binding capacity of blood (Ots, et al. 1998). The exposure to lead is directly associated with the inhibition of enzyme δ-aminolevuline acid dehydratase (ALAD), which affects the formation of hemoglobin (Papanikolaou, et al. 2005). Zinc is an essential element to perform the normal functioning of ALAD, but lead replaces this element, which affects the normal functioning of this enzyme. ALAD is a useful biomarker for the study of metal contamination and its effects. Several studies concluded that cadmium and lead cause anemia by decreasing the level of hemoglobin, hematocrit, and mean cell volume (Iolascon, et al. 2009). Cadmium and lead are considered as most dangerous heavy metals, which come into the environment by burning fossil fuels, vehicles, and industries (Kenntner et al., 2003). Several previous studies revealed that lead and cadmium are very toxic for avian fauna such as causing abnormalities in endocrine system, molting, reproduction, growth rate, and enzymes involved in hemoglobin formation (Cheney et al., 1981; Eisler, 1988; Honda et al., 1986). Nickel is used as a catalyst in different industries is deposited into the natural water bodies, which are taken up by different organisms and damage their respiratory organs and DNA.

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Zinc is used in various kinds of industries such as cosmetic industry, electric industry, and agrochemical industry causes nausea, stomach disorders, and fainting (Peplow, 2000; Pérez-López et al., 2008). In south Asia, the metal pollution has increased in a few years (Hardoy et al., 1992).

Due to the increasing metal pollution and its adverse effects, we need to assess the environmental status, but we cannot estimate all of the parts of the environment, so we can use a bio-indicator, which represent the environmental conditions (Brait and Antoniosi Filho, 2011; Falq et al., 2011; Scheifler et al., 2006). The appropriate bio-indicator is required to estimate the concentration and hazardous effects of these metals in the environment. The bio-indicators are living organisms, which represent their environmental status correctly (Burger and Gochfeld, 2007; Kalisińska et al., 2004). Avian species are considered as an important and appropriate bio-indicator still from 1960s. Birds are considered as good bio-indicators because they feed on diverse types of food and can represent primary fluctuations in food chain (Boncompagni et al., 2003). Avian species can be used to estimate environmental contamination because they are widely distributed in the environment and take food from different types of sources (Furness, 1993; Llabjani et al., 2012). The use of the internal organs of birds has proven to be a good bio-indicator, but it is a destructive method because of many ethical and conservative issues related to the use of internal organs (Jaspers et al., 2005; Van den Steen et al., 2006). Birds are liable to environmental pollutants either from contaminated air, drinking water, and dietary intake. The metals accumulate in the body and become the part of bloodstream, and metals are integrated with the keratin structure in the feathers (Tsipoura et al., 2008). In Europe and United States, avian species are used as bio-indicator to estimate environmental pollution (Burger 2013; Frantz et al., 2012). Feathers can be used as an alternative bio-indicator without sacrificing the bird because they are easy to collect, transport and store; moreover, feathers can be collected without affecting the fitness of birds (Spahn and Sherry, 1999).

The estimation of metal pollution does not give us any information about the biological stress ccurring due to metal contamination. Therefore, we need an approach to estimate the negative effects of metal contamination on health parameters of avian fauna (Peakall, 1992). The assessment of hematological parameters can be used to assess the health status and biological stress. The normal hematological status is compulsory for the survival of avian fauna (Kilgas et al., 2006; Nadolski et al., 2006). Many birds do not show the symptoms of diseases at early stages of illness. Hematological parameters play an important role to evaluate the health status, so we can recognize many diseases at early stages by the use of hematological assays (Scope et al., 2005). Many features are related with hematological values such as source of food, day length, molting period, and environmental conditions (Millaku et al., 2000). The estimation of metal contamination is very useful tool to assess the environmental status. The evaluation of health risks associated with metal contamination is very necessary for the conservation of flora and fauna (Herrera-Duenas et al., 2014). The abnormalities in hematological parameters are associated with breeding capabilities of avian species such as low level of hematocrit associated with low body conditions (Svensson and Merila, 1996), infectious diseases by parasites of blood (Booth and Elliott, 2002), and also flying abilities (Saino et al., 1997).

Like other developing countries, Pakistan is also facing the problem of population development, urbanization, and industrialization in recent years, which has resulted in environmental contamination (Khan, 1991; Qadir et al., 2008; Zaman and Ara, 2000). The contamination of environment by metal pollutants is more in industrial cities (Hashmi et al., 2013). The use of agrochemicals, fertilizers, and industrial effluents are the major sources of metal contamination in Pakistan (Baluch, 1995). Several studies revealed higher concentration of heavy metals in water bodies of Pakistan (Tariq et al., 1994; Tehseen et al., 1994). Punjab is most developed, urbanized, and industrialized province of Pakistan. During the last few years, the environmental pollution has increased due to human activities. The industrial and agricultural activities are dangerous for water bodies, fishes, birds, soil, and sediments (Eqani et al., 2012; Hashmi et al., 2013; Qadir and Malik, 2011; Syed and Malik, 2011). Sialkot city is internationally famous for its sports, leather, and surgical industries. The effluents, solid wastes, and air particles emitted from leather industries are the major sources of environmental contamination. The leather industries use seventeen different types of tanning agents, but chromium usage is ninety percent (Iqbal et al., 1998). This research was conducted to estimate the concentration of metals and the negative impact of metals contaminants on hematological parameters in the selected avian fauna from Sialkot to assess the environmental contamination and biological stress resulted due to the metal contamination.

2. Materials and methods

2.1. Study area

Samples were collected from different sites of Sialkot, Pakistan. In the last decade, due to increase in population size of the city, development of industries has taken place. These include tanneries, steel factories, leather industry, garments industry, and sports industry. Sialkot generated nineteen million-meter cubes per year of wastewater. There is no water treatment facility available in Sialkot, and this water is drained into natural water bodies.

2.2. Sampling

Fifty-eight birds were captured from different sites of Sialkot by using mist net with mesh size of 30–38 mm (Gushit et al., 2016). The chest feathers samples were collected from house sparrow (n = 21), common myna (n = 24) and common quail (n = 13) and stored in a paper envelop for the analysis of metal contaminants. Twenty-seven blood samples were collected by using 1mL syringe from the jugular or ulnar vein of house sparrow (n = 9), common myna (n = 11) and common quail (n = 7) and then shifted into EDTA containing vacutainer for hematological and metal contents analysis. The blood samples were stored at 4 °C immediately (Geens et al., 2010). All of the methods, which were used in this study, are approved by the ethical committee of the University of veterinary and animal sciences Lahore-Pakistan.

2.3. Metal analysis

The feather samples were washed with tap water and then cleaned with distilled water and acetone (3 times) for the removal of any external contaminants. The feather samples were air dried before drying in an oven. Feather samples transferred into 4 mL polypropylene vials and dried in oven for 24 h at 60 °C. The feathers were cut into small pieces by using stainless steel scissor to allow easy digestion with acid. The 0.01 mg dry weight of feather samples was calculated by digital balance. The remaining blood samples after hematological analysis were taken in polypropylene vials. The blood samples were dried in an oven at 60 °C for 24 h. A 100 Ml stock solution was prepared with a (1:1) ratio by volume with a mixture of seventy percent HNO3 (Merck, Germany) and thirty percent H_2O_2 (Merck, Germany), respectively per 10 µg dry sample. The digestion of samples by microwave heating continued until the mixture became pale yellow. The completely digested samples were filtered and diluted with 2 mL of double distilled water (MilliQ, Milipore). The samples were stored at –20 °C in a sample bottle before further analysis. The concentrations of chromium, nickel, copper, zinc, cadmium and lead were analyzed by atomic absorption spectrophotometer (SHIMADZU AA-7000F/AAC). The limits of quantification for all metals were set at 0.01ug/l. For quality control blanks and certified reference material (VMK 102, Bureau of Certified References) were used in analyses. The concentration of metals in certified samples were within 10% of the certified values. The concentrations of metals were represented on fresh weight basis in µg/g (Geens et al., 2010).

2.4. Hematological analysis

The following hematological parameters were analyzed: the number of erythrocytes (RBC in 10^6/µL counting of leucocytes (WBC in 10^3/µL), differential leukocytes count, mean cell volume (MCV in fl), mean cell hemoglobin (MCH in pg), and mean cell hemoglobin concentration (MCHC in g/dL) by using automated hematology analyzer NIHON KOHDEN, MEK-6550.

2.5. Statistical analysis

SPSS software was used to analyze data. The descriptive analysis was used to find out the means and standers error. One way analysis of variance ANOVA was applied to check the significance level for mean values among different species. The significant differences were analyzed by performing Tukey HST test. Pearson correlation was performed to find out the relationship between metals in feathers and blood. The linear regression was performed to estimate the correlation between metals in blood and hematological parameters. $P < 0.05$ was considered significant for all analyses.

3. Results

3.1. Metals concentration

The results of this study revealed significant variations for concentrations of metals in feathers between different species. The concentrations of copper and nickel in blood not varied significantly, but all other metals vary significantly among house sparrow, common myna, and quail. The highest concentrations of lead, chromium, nickel, and copper in feathers were examined in common myna. The Quail revealed highest concentration of cadmium and zinc both in feathers and blood. The highest concentration of chromium and lead were found in blood of common myna. The mean concentrations of metals in blood and feathers with ANOVA significance are given in (Table 1). The concentrations of metals at different study spots in feather samples are represented in Figure 1 and the ones in blood samples are represented in Figure 2. We also find the relationship between metal levels in feather and blood samples by performing Pearson correlation. The results of this study founded a significant positive association between Cu ($r = 0.972$, $p = 0.000$), Zn ($r = 0.979$, $p = 0.000$), Ni ($r = 0.761$, $p = 0.001$), Cd ($r = 0.897$, $p = 0.000$) Cr ($r = 0.911$, $P = 0.006$) and Pb ($r = 0.879$, $p = 0.001$) levels in blood and feather samples of avian species.

3.2. Hematological parameters

The one way analysis of variance revealed the significant variation in hematological parameters among house sparrow, common myna, and quail. The mean concentrations of hematological parameters and ANOVA significances are given in (Table 2). The highest concentration of RBCs, MCH, and MCHC were observed in quail than other species. The level of WBCs was higher in common myna. The sparrows revealed the highest concentration of MCH among three species. The results of this research did not show significant variations between house sparrow and common myna; however, both were significantly different from common quail in terms of the level of red blood cells ($p = 0.000$, Tukey HST test p

Table 1. Mean concentration (µg/g fresh weight ± S.E) and one way ANOVA results for metal contents in feathers and blood samples.

Metals	Sample	Species			ANOVA Sig.
		House Sparrow (*Passer domesticus*)	Common Myna (*Acridotheres tristis*)	Quail (*Coturnix coturnix*)	
Copper	Feathers	19.49 ± 1.59[a]	23.95 ± 2.28[a]	11.87 ± 1.42[b]	0.002**
	Blood	0.62 ± 0.21[a]	0.63 ± 0.19[a]	0.22 ± 0.04[a]	0.200
Zinc	Feathers	72.85 ± 5.87[b]	66.74 ± 5.90[b]	139.63 ± 12.89[a]	0.000**
	Blood	6.02 ± 0.50[b]	5.18 ± 0.81[b]	14.18 ± 1.67[a]	0.000**
Nickel	Feathers	7.09 ± 0.71[ab]	8.69 ± 1.25[a]	3.87 ± 0.74[bc]	0.011*
	Blood	0.47 ± 0.22[a]	0.68 ± 0.23[a]	0.43 ± 0.18[a]	0.696
Cadmium	Feathers	1.52 ± 0.29[b]	1.92 ± 0.33[b]	5.53 ± 0.85[a]	0.000**
	Blood	0.30 ± 0.18[b]	0.11 ± 0.04[b]	1.32 ± 0.14[a]	0.000**
Chromium	Feathers	24.38 ± 2.85[b]	45.35 ± 2.69[a]	16.47 ± 2.28[b]	0.000**
	Blood	1.80 ± 0.15[b]	2.92 ± 0.37[a]	1.11 ± 0.20[b]	0.001**
Lead	Feathers	9.16 ± 1.17[a]	14.93 ± 1.54[a]	4.45 ± 0.63[b]	0.000**
	Blood	0.36 ± 0.11[b]	1.30 ± 0.20[a]	0.29 ± 0.17[b]	0.002**

< 0.05) and mean corpuscular hemoglobin concentration ($p = 0.00$, Tukey HST test $p < 0.05$). Common myna and common quail did not reveal significant variations, while both varies significantly from house sparrow in terms of the numbers of white blood cells ($p = 0.007$, Tukey HST test $p < 0.05$) and mean cell volume ($p = 0.000$, Tukey HST test $p < 0.05$). We also estimated the percentage of differential leukocyte count in white blood cells. The house sparrow and common myna shows significant differences with quail for the percentages of lymphocytes, monocytes, heterophils, and basophils as shown in (Table 3).

3.3. Relation between metal pollution and hematological parameters

The results of this research not reported significant correlation between metals concentrations in blood and hematological parameters. However, we find some negative correlations between blood metal contents level and hematological parameters. The cadmium showed negative relation with WBCs and MCV. The results revealed negative association between MCV, MCH, and chromium. The lead showed negative relation with all hematological parameters except white blood cells. According to ANOVA results of linear regression, white blood cells revealed significance positive correlation with metal contaminants. The results of correlation between metals and hematological parameters are given in (Table 4).

4. Discussion

Copper is required in small amount to perform some normal metabolic activities. However, the exposure of copper in large amount may lead to serious damages such as abnormalities of the reproductive system, respiratory system, digestive system, and endocrine system. The accumulation of copper in large amount may also damage blood cells and may cause cancer (Chen et al., 1993). The main sources of copper in the environment are mining activities, sewage sludge, and agricultural activities such as usage of fungicides (Ullah et al., 2014). The results of this study concluded that the concentration of copper in blood and feathers were present in the following descending order between three species common myna > house sparrow > quail. According to analysis of variance the concentration of copper in feathers varies significantly between species. However, the results for the concentration of copper in blood do not vary significantly between house sparrow, common myna, and quail. The level of copper was not differing significantly between feathers of sparrow and common myna, but sparrow and common myna revealed significant variations with quail. According to our research, the increased level of copper in blood results in increased level of copper in feathers. The concentration of copper not revealed significant relation with hematological parameters. The results of house sparrow for concentration of copper in feathers were comparable with the study of Manjula et al., 2015; Swaileh and Sansur, 2006. The copper concentration in blood samples of sparrow and common myna were comparable with the one (Lester and van Riper, 2014) in blood of song sparrow. The results of (Manjula et al., 2015) for rural area were comparable with the copper contents in common myna used in the present study; however, the concentration of copper in urban area were higher than the one found in the present study. The

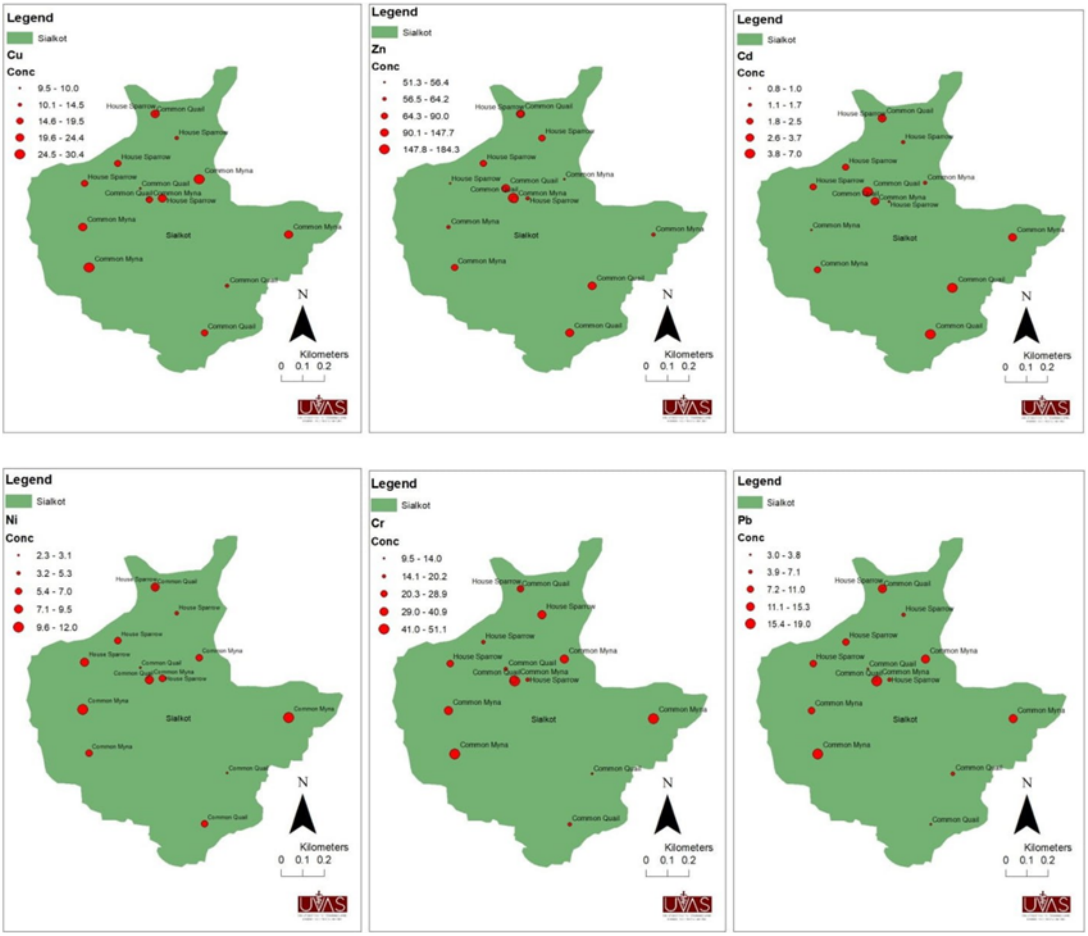

Figure 1. Maps showing concentrations of metal (ug/g) in feather samples on different sampling sites of Sialkot.

concentration of copper in our study was higher than the results of (Abbasi et al., 2015; Mustafa et al., 2015).

Zinc is an essential element for many metabolic reactions. The zinc originated in environment as a result of both human and natural activities. The burning of solid wastes, construction activities, and marble industries are also sources of zinc pollution (Ullah et al., 2014). The usage of zinc salts is the major source of zinc contamination in our study area (Abdullah et al., 2015). There are no sides effects of zinc are known, but some studies reported 1200 µg/g of Zn as threshold limit (Taggart et al., 2009). The highest level of zinc was founded in quail birds between three species. The level of zinc contents was different significantly between house sparrow, common myna, and quail. According to our estimations, zinc does not affect significantly hematological parameters. However, zinc only showed positive association with white blood cells. The concentration of zinc in house sparrow and common myna feathers was comparable with the results of Manjula et al., 2015; Abbasi et al., 2015. The results of our study detected higher concentration of Zn in house sparrow than the concentration found by Mustafa et al., 2015; Swaileh and Sansur, 2006. The uptake of zinc in common myna feathers were higher in the study of Muralidharan et al., 2004. The zinc level in quail was almost similar with the results of Malik and Zeb, 2009, which were reported for cattle egret. The level of zinc in our blood samples was too much lower than the results of Bounagua et al., 2014. However, the zinc level in blood samples of house sparrow and common myna were similar with the results of Geens et al., 2010 for great tit and Lester and van Riper, 2014 for song sparrow. However, zinc was found at safe level in our study samples. The nickel exposure may cause some allergic reactions, damages to bronchitis, digestive abnormalities and as well nickel is a carcinogen (Abdullah et al., 2015). The industrial activities such as ghee production, batteries manufacturing, leather production, and Ni-Cr plating are the major reasons of nickel contamination (Ullah et al., 2014). According to (Outridge and Scheuhammer, 1993) the birds from unpolluted area accumulate nickel in range of 0.1–5 µg/g. The level of nickel was quite different in feather samples between three species, but, in blood

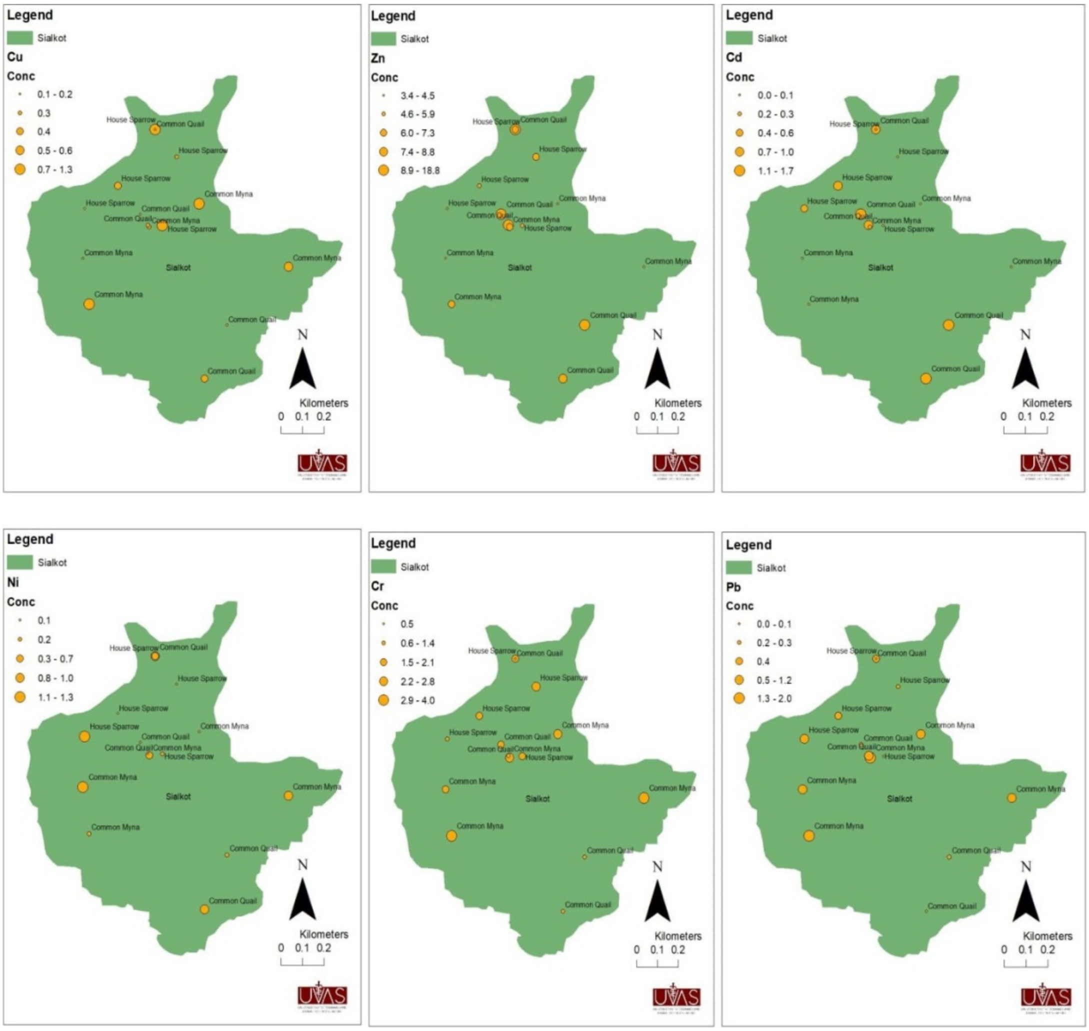

Figure 2. Maps showing concentrations of metal (ug/g) in blood samples on different sampling sites of Sialkot.

samples, there were no major differences between species. Nickel revealed negative correlation with cadmium and zinc; however, it is positively correlated with all other metals, which were under observations for both in blood and feather samples. There were not any significant association between nickel and hematological parameters. The concentration of nickel in house sparrow and common myna feathers showed similarities with the results of Manjula et al., 2015 for house sparrow and common myna (Geens et al., 2010) for Great tit and (Malik and Zeb, 2009) for cattle egret. The quail feather samples accumulate small amount of nickel than house sparrow and common myna according to our estimation. The blood samples of all species revealed slightly higher level of Ni than the findings of (Geens et al., 2010).

Both the natural and human activities are the major sources of cadmium contamination such as soil erosion and metallurgical activities. Some commercial products are also source of cadmium pollution such as plastic stabilizer, batteries, paints, and devices coated with metals (Malik et al., 2010; Qadir and Malik, 2011). The accumulation of cadmium takes place in the body of organisms with the passage of time. The higher concentration of cadmium causes eggshell thinning, damages of kidney and abnormities of calcium metabolism, reproductive system, and digestive system (Burger, 2008). The concentration of cadmium among species were founded in following descending order quail > common myna > house sparrow. The concentrations of cadmium in quail blood and feathers were significantly different from sparrow and common myna. According to our results, cadmium was not significantly associated with hematological parameters. However, we find some negative relation between cadmium and mean cell volume and white blood cells. The results for the cadmium concentration in sparrow and common myna were almost similar with the results of (Lester and van Riper, 2014; Manjula et al., 2015; Muralidharan et al., 2004; Mustafa et al., 2015). The level of cadmium in blood

Table 2. Results of one way ANOVA means and standard errors of hematological parameters.

Parameters	Species			ANOVA Sig.
	House Sparrow (*Passer domesticus*)	Common Myna (*Acridotheres tristis*)	Quail *(Coturnix coturnix)*	
RBCs (10^6/µl)	1.34 ± 0.06^b	1.87 ± 0.02^b	4.678 ± 0.70^a	0.000**
WBCs (10^3/ µl)	14.79 ± 0.71^b	23.80 ± 0.71^a	21.52 ± 2.76^a	0.007**
MCV (fl)	168.64 ± 3.93^a	141.32 ± 1.37^b	128.54 ± 6.14^b	0.000**
MCH (pg)	68.62 ± 2.70^{ab}	56.67 ± 1.10^b	72.90 ± 5.66^a	0.023*
MCHC (g/dl)	40.93 ± 1.02^b	38.80 ± 1.20^b	64.29 ± 7.52^a	0.003**

Table 3. Results of differential leukocytes count in percentage and one way ANOVA.

Parameters	Species			ANOVA Sig.
	House Sparrow (*Passer domesticus*)	Common Myna (*Acridotheres tristis*)	Quail (*Coturnix coturnix*)	
Lymphocytes	41.41 ± 1.07^b	44.43 ± 2.34^b	63.46 ± 2.48^a	0.000**
Monocytes	1.40 ± 0.03^b	1.49 ± 0.02^b	2.63 ± 0.36^a	0.002**
Heterophils	38.51 ± 2.00^a	37.04 ± 1.70^a	22.50 ± 4.45^b	0.004**
Eosinophils	18.31 ± 1.77^a	16.92 ± 1.81^a	10.40 ± 3.88^a	0.126
Basophils	0.58 ± 0.01^b	0.54 ± 0.02^b	1.59 ± 0.38^a	0.009**

Different Alphabetic letters indicate significant differences in metal levels among species derived through post-hoc (tukey) test.

Table 4. Relation between concentration of metals in blood and hematological parameter.

Independents	Dependents				
	RBCs	WBCs	MCV	MCH	MCHC
Cu	0.440	0.846	0.999	0.578	0.593
Zn	0.282	0.011*	–0.058	–0.058	0.352
Ni	0.116	0.531	0.400	0.360	0.135
Cd	0.053	–0.621	– 0.300	0.139	0.165
Cr	0.625	0.816	–0.231	–0.484	0.909
Pb	–0.384	0.006*	–0.629	–0.192	–0.202
ANOVA Sig.	0.059	0.009**	0.136	0.076	0.073

and feather samples of house sparrow and common myna were lower than the study of (Geens et al., 2010) reported from contaminated areas for great tits and (Malik and Zeb, 2009; Ullah et al., 2014) reported from Pakistan for cattle egret. The permissible range of cadmium for birds is 2µg/g (Burger and Gochfeld, 2000). The level of cadmium in quail feathers was slightly greater than the permissible range.

Chromium is a micro essential element and may cause hazardous effects on reproductive system (Malik and Zeb, 2009). The major source of chromium contamination in our study area is leather industry, which use various chromium salts as a tanning agent (Qadir et al., 2008). Various avian species secret chromium salts through nasal glands, which can bind with the keratin of feathers. The highest accumulation of chromium was recorded in common myna during our findings. The common myna revealed significant variations with quail and house sparrow for the concentration of lead in feathers and blood. We did not find significant negative impact of

Table 5. Comparison of metals (μg/g) in feathers and blood between sparrow, common myna, and quail from previous and present study.

Authors world wide	Species	Sample	Study area	Zn	Cu	Ni	Cd	Cr	Pb
(Swaileh and Sansur, 2006)	House sparrow	Feathers	Palestine, Qalqilia, Ramallah city	54.9	19.5		0.02		8.1
(Manjula et al., 2015)	House sparrow	Feathers	India Tiruchirapalli (rural area)	75.91	21.04	8.13	1.71	55.4	
		Feathers	India Tiruchirapalli (urban area)	98.16	148.29	76.6	1.64	53.1	
(Bounagua et al., 2014)	House sparrow	Blood	Morocco (Abdullah *et al.*)	331.07			3.78		35.42
		Blood	Morocco (Town center)	326.12			5.63		40.12
		Blood	Morocco (Agdal)	212.74			3.88		32.03
		Blood	Morocco (Oulja)	284.6			8.34		33.72
		Blood	Morocco (Bahraoui	403.7			3.74		2.7
(Manjula et al., 2015)	Common myna	Feathers	India Tiruchirapalli (rural area)	65.1	25.85	7.54	1.59	55.1	
		Feathers	India Tiruchirapalli (urban area)	70.65	80.32	8.51	1.7	85.7	
(Muralidharan et al., 2004)	Common myna	Primary feathers	India Nilgiris	137.98	1.86		1.89	0.58	2.2
		Secondary feathers		179.68	5.69		3.08	0.27	
Pakistan									
(Mustafa et al., 2015)	House sparrow	Feathers	Sargodha	14.78	0.14		1.81		2.67
(Abbasi et al., 2015)	Common myna	Feathers	Baroghil valley	60.83	1.04	1.73	0.35	0.6	0.95
			Soanvally	91.05	2.19	1.88	0.78	1.11	2.15

chromium pollution on hematological parameters, but the results revealed some negative association between chromium contamination with mean cell volume and mean cell hemoglobin constantans. The concentration of chromium in our feathers and blood samples were greater than the findings of (Lester and van Riper, 2014) reported for song sparrow from Arizona and of (Muralidharan et al., 2004) reported for common myna from India. Our results revealed slight similarities with the estimations of (Ullah et al., 2014) reported for cattle egret from Pakistan. According to (Burger and Gochfeld, 2000) the permissible range of chromium in birds is 2.8μg/g. So, the concentrations of chromium in all studied species were greater than safe level, which can exert negative impact on the health status of avian species.

Lead is a highly toxic element; the major sources of lead in environment are vehicles, burning fossil fuel, paints, furniture polishing, and tanning industries. The lead is not required for any metabolic process, and lead showed great affinity to accumulate in feathers and calcareous tissues (Jerez et al., 2011; Markowski et al., 2013). According to the study of Burger and Gochfeld, (2000) the level of lead more than 4μg/g leads to the abnormalities in thermoregulation, locomotion reproductive system, and feeding behavior. The threshold value of lead in blood is 2μg/g (Geens et al., 2010). The accumulation of lead in feathers and blood samples were founded in the following descending order common myna > house sparrow and quail. There were significant differences occurred between different species for lead accumulation. There was no significant relation founded between lead concentration and hematological parameters, but we find some negative relations with hematological parameters except white blood cells. The increased level of lead results in decreases in hematological parameters. The lead concentrations in other studies reported from Pakistan and other parts of the world for

Table 6. Comparison of metals (µg/g) in feathers and blood of different species with present study.

Author world wide	**Species**	**Sample**	**Study area**	**Zn**	**Cu**	**Ni**	**Cd**	**Cr**	**Pb**
(Geens et al., 2010)	Great tit	Feathers	South Antwerp (UM)	240	37	13	11		140
			South Antwerp (F8)	216	36.5	14	17		110
			South Antwerp (F7)	142	9.8	7.2	0.39		8.1
			South Antwerp (F4)	184	9.8	5.7	0.19		4.1
		Blood	South Antwerp (UM)	5.5	0.13	0.11	0.016		0.28
			South Antwerp (F8)	6.9	0.09	0.14	0.011		0.17
			South Antwerp (F7)	7.9	0.2	0.13	0.007		0.03
			South Antwerp (F4)	6.9	0.13	0.18	0.007		0.02
(Lester and van Riper, 2014)	Song sparrow	Blood	Arizona (LOCH)	195.1	11.69	1.22	0.109	0.418	0.706
			NOWA	9.606	0.811	0.033	0.005	0.047	0.031
			PLSP	6.425	1.118	0.043	0.004	0.023	0.039
			SOCR	6.831	0.578	0.035	0.002	2.145	0.031
			NIWWTP	7.509	0.699	0.083	0.004	0.12	0.108
			TUMA	8.219	0.74	0.025	0.004	0.018	0.027
		Feathers	LOCH	7.132	0.672	0.136	0.002	0.035	0.041
			NOWA	215.6	15.35	1.144	0.362	0.431	1.191
			PLSP	196.3	11.4	0.752	0.366	0.238	2.283
			SOCR	189	10.86	0.736	0.127	0.387	2.273
			NIWWTP	206.3	11.87	3.457	1.022	0.763	1.069
			TUMA	171.6	10.73	1.323	0.422	0.364	1.05
Pakistan									
(Malik and Zeb, 2009)	Cattle egret	feathers	chenab	133.8	4	9.1	3.1	6.6	37.5
			ravi river	155.2	3.7	8	2.4	7.1	76.5
			raval lake	138.4	4	7.8	2.7	5.38	60.2
Ullah et al., s 2014)	Cattle egret	feathers	Trimium headwork	18.5	0.9	0.1	3.3	26.2	30
			Shorkot	18.8	0.2	0.2	3	30.8	32.5
			Mailsi	10.7	0.2	0.2	1.7	35.8	43.1

house sparrow feathers (Mustafa et al., 2015; Swaileh and Sansur, 2006), and for common myna feathers (Abbasi et al., 2015; Muralidharan et al., 2004) were lower than our measured concentrations. Malik and Zeb, 2009 and Ullah et al., 2014 reported higher concentrations of lead than our results in cattle egret feathers from Pakistan. The results for lead in blood were slightly higher in our studied species than the findings of Lester and van Riper, 2014. The level of lead in common myna and house sparrow feathers and blood was not at safe level according to the threshold limits of Burger and Gochfeld, 2000; Geens et al., 2010.

The feathers accumulate highest concentration of metals than blood; the most probable reason behind that is the feathers reflect exogenous contamination, and blood only represent the immediate dietary sources of contamination (Veerle et al., 2004). So, the feathers can be used as an appropriate approach to find out the environmental contamination level. The major reason of differences between different species was difference in feeding habitat (Abbasi et al., 2015). The house sparrow and common myna are omnivores, but house sparrow mostly feed on grains. The quail is less mobile and feed 95% on plants so the metal contamination was founded at fewer rates in quail bird. So, the metal contamination was founded in the following descending order common myna> house sparrow> quail. The comparison of our study with other studies at Pakistan and international level are given in (Table 5, 6).

The hematological parameters were significantly varied between species. According to our results there were no significant or non-significant negative association observed between copper and Zinc concentrations in blood and hematological parameters. The results of (Samanta et al., 2011) for broiler chicken revealed that dietary copper more than 150mg/Kg negatively reduce the concentration of hemoglobin. However, there were no effect of copper were observed on total leukocyte count and different leukocyte count. The study of (Aksu et al., 2010) concluded that there are no negative effects of copper so the appropriate concentration of copper from organic sources can be used in the diet of broilers. According to (Dönmez et al., 2002) the level of hemoglobin, hematocrit and erythrocyte, leukocyte is not affected by exposure of zinc in broiler chicks. (Gupta et al., 1985) reported that the leukocyte count increase in rats by supplementation of zinc in diet. However, Donmez and Keskin, 1999 report no significant positive or negative effects of zinc on leukocyte count in rats and goat. Our results concluded that the cadmium was negatively correlated with MCV and WBCs; however, the numbers of RBCs, MCH, and MCHC were increased by increasing cadmium level. The results of (Gabol et al., 2014) can be compared with our study for the impact of cadmium on hematological parameters. The study of (Ali et al., 2020; Gutyj et al., 2019) represent opposite results with our research which concluded a negative relation of cadmium exposure with HGB, MCHC and RBCs while show positive relation with WBCs. The dietary sources of chromium may have positive relation with hematological parameters in Japanese quails which reared under heat stress conditions (El-Kholy et al., 2017). Nickel does not show any significant association with hematological parameters. The results revealed that the chromium and lead were negatively correlated with hematological parameters and chromium and lead were not founded at safe level, which can exert negative impact on health status of birds. The highest level of chromium and lead were observed in common myna, so hematological parameters were most affected in common myna as compared to house sparrow and quail. However, the level of white blood cells was significantly increased by increasing heavy metals in blood. The results for differential leukocyte count showed significant variations among different species except eosinophils. The increased level of chromium causes decreased numbers of MCH and MCV. The chromium is positively associated with the concentration of red blood cells and mean cell hemoglobin concentration. Some other studies also revealed positive association between chromium and red blood cells such as (Cui et al., 2005; Gabol et al., 2014). We founded that by increasing lead concentration, the number of RBCs, MCV, MCH and MCHC decreased, but the numbers of WBCs increase. Various previous studies (Gabol et al., 2014; Geens et al., 2010) founded the negative impact of lead on hematological status of avian species. The accumulation of lead causes anemia in birds by breaking down of blood cells (Katavolos et al., 2007). The lead damage red blood cells and causes break down of plasma membrane (Leggett, 1993). ALAD activity, hematocrits level, and hemoglobin concentration decreased by increasing lead concentration (Cid et al., 2018). Several changes in blood cells such as erythrocytes agglutination, nucleus displacement in erythrocytes, absence of nucleus in erythrocytes, lymphocytes enlargement, immature erythrocytes, rotation of nucleus, large macrophage, large size lymphocytes, and aggregation of thrombocytes were examined in Japanese quails, which were exposed to lead. The numbers of healthy erythrocytes, lymphocytes, and heterophils were decreased, and there were no significant changes observed in numbers of leucocytes by exposure of lead chloride (Suljević et al., 2020). The study of Rogival et al., 2006 revealed negative effects of lead and cadmium exposure on hematological parameters in wood mice. The study of Li et al., 2021 reported that the air pollutants are negatively associated with hematological parameters.

According to our estimations among three species common myna were most affected by metal contamination. The findings of our research were concluded that the feathers of avian species can be used as an efficient bio-indicator to assess the metal contamination level in an environment; however, feathers did not revealed the internal contamination level. The results revealed that the hematological parameters were affected by increasing metal contents, especially lead and chromium. Therefore, the assessment of hematological parameters can be used to find out the negative impact of metal contamination on health status. We concluded from our research that the level of chromium and lead were not at a safe level, and they have exert negative impact on health status in avian fauna of Sialkot. The findings of this study will be helpful in environmental management and to understand the environmental status of study area.

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