Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 2 Year 2024 Page 1451-1457

Hematological Variations And Assesment Of Heavy Metals In Sindh Sparrow (Passer Pyrrhonotus) As A Bioindicator In Punjab Areas Of Pakistan

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ABSTRACT

Sindh sparrows, prevalent in Sindh region of Pakistan, are non-migratory birds abundant in human-inhabited areas globally, except Antarctica. Given their close association with human settlements, these birds serve as crucial indicators for biological monitoring in regions affected by pollution. This study focused on assessing the hematological parameters and heavy metal concentrations in various organs of Sindh sparrows in the Punjab regions of Pakistan. The collected blood samples underwent analysis for hematological indices and heavy metal concentrations. The results indicated variability in hematological analysis of sparrows. Fluctuations were observed in the concentrations of different heavy metals, with significant differences in zinc and iron, while chromium, cadmium, and nickel concentrations showed non-significant differences. The patterns of heavy metal accumulation revealed higher concentrations of chromium, nickel, and iron in the liver, contrasting with the cadmium and zinc in kidneys. In conclusion, significant correlations were observed among the concentrations of Zinc, Chromium, Cadmium, Nickel, and Iron in kidney, liver, and muscles of both male and female Sindh sparrows. The findings underscore the need for further comprehensive studies to assess heavy metal accumulation in birds, highlighting the urgency to address environmental pollution that poses risks to avian species and human populations worldwide.

Keywords**: **Sindh Sparrow, Avian species Hematology, Heavy metals, Pakistan

INTRODUCTION

The House sparrow (Passer domesticus Linnaeus, 1758) and Sindh sparrow (P. pyrrhonotus Blyth, 1844) (Passeridae: Passeriformes) are non-migratory birds in Sindh, exhibiting striking similarities in their appearance [1, 2]. These sparrow species are globally abundant, with the exception of Antarctica, and are

closely associated with permanent human habitations such as farmyards, villages, parks, suburban areas, and city centers [3]. The genus Diplotriaena Railliet and Henry, 1909 has been documented in over 60 species from various birds worldwide [4-6]. In Pakistan, D. nochti was identified in the Rosy Starling Sturnus roseus, Bank Myna Acridotheres ginginianus, and D. streptopelia was observed in the Laughing Dove Streptopelia senegalensis [7].

Hematology plays a crucial role in assessing the health status of the Sindh Sparrow. Many Sindh Sparrows do not exhibit measurable indicators until later stages of disease progression, and the observed signs may be subtle and nonspecific. In avian veterinary practice, the utility of blood tests as essential diagnostic tools is widely recognized. Nevertheless, the implementation of blood tests, the acquisition of adequate samples, and the interpretation of results continue to pose challenges in the case of free-ranging Sindh Sparrows. Hematological parameters may be influenced by factors such as the time of day, molting period, nutritional status, and environmental conditions [8].

Controlling the accumulation of hazardous substances in ecosystems holds significant importance within the context of global atmospheric pollution. The merits of employing biological monitoring are readily apparent [9]. Radiationally, birds have served as focal points for biological monitoring in polluted ecosystems, particularly in areas neighboring stationary pollution sources. Extensive research has been undertaken on the concentration of heavy metals in birds, notably in Silesia, one of Europe's most polluted regions [10, 11], North Europe [12], and America [13]. While studies on the accumulation of toxicants in bird populations exist for specific regions in Russia [14, 15], comprehensive environmental quality monitoring is widespread. Notably, there is a dearth of research on hematology and heavy metal pollution in Sindh sparrows in Pakistan to date. Therefore, this study was designed to explore hematological parameters and heavy metal concentrations in various organs of Sindh sparrows in the Punjab regions of Pakistan.

MATERIALS AND METHODS

Site selection and study duration

Samples were gathered from various water body locations, including lower Baari Doab, LBD Chuchak Road, Tehsil Renala, Head Sulimanki, and Bahawalnagar, spanning Districts Okara and Bahawalpur. These areas serve as vital watering spots for migratory birds. During the winter season, migratory birds travel from India to Pakistan over the Satluj River in search of food. Head Sulemanki (29.49 NL, 72.33 E; altitude 177 m above sea level), situated in the southeast of Punjab on the Satluj River, receives water from the Ravi River and Chenab, making it a significant location for migratory birds. The brackish water bodies of Bahawalnagar (29.59 N, 73.16 E) offer both feeding and resting grounds for migratory birds. The LBD canal, which receives water from the Ravi River, serves as a substantial site for the feeding and resting activities of birds. These sites were visited daily during the morning and evening from December 2020 to March 2021.

Ethical concern and consent to publish

The research adhered to the principles outlined in the Declaration of Helsinki. Ethical clearance was secured from the university administration to carry out the study, and formal consent was obtained from the relevant department for the publication of the findings

Blood Samples collection and hematological analysis

During the fieldwork, 20 blood samples were collected from Sindh sparrows, with approximately 10ml of blood obtained from each adult bird during field visits. The blood collection process involved gently and properly capturing the Sindh sparrows, adhering to safety rules, and puncturing the jugular vein using disposable syringes [16]. Stringent animal ethics were ensured throughout the procedure. The blood was collected using syringes, with one glass tube containing ethylene-diamine-tetra-amine (EDTA) as an anticoagulant for hematological analysis, and the other tube without EDTA for blood serum analysis. The laboratory analysis involved specific experimental techniques, and various hematological parameters were identified [17]. Hematological analysis for heparinized blood samples included Total Leukocyte Count (TLC), Packed Cell Volume (PVC), Total Erythrocyte Count (TEC), Hemoglobin (Hb), Differential Leukocyte Count (DLC), and Packed Cell Volume (PCV), utilizing an automatic hematological analyzer XP-100 Sysmex from Japan. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined from erythrocyte series values [18].

Heavy Metal analysis

The captured Sindh sparrows were euthanized by asphyxiation, temporarily stored in a refrigerator, and then transported via airfreight in a cooling box. A total of 30 samples, consisting of liver and kidney tissues, were collected from different individuals. The liver and kidneys were dissected and promptly weighed. Subsequently, the kidney, muscle, and liver tissues were dried in an oven at 100 degrees Celsius [19]. The dried samples were ground into a powder form.

To analyze traces of metals in the samples, 1.0 g of each sample was ingested in a conical flask with 10 ml of HNO3 and 15 ml of H3PO4. The solution in the conical flask was left overnight to digest at room temperature. On the following day, 2 ml of hydrogen peroxide (H2O2) was added to the samples, and the mixture was heated up to a temperature of 120 degrees Celsius on a hot plate. The samples were then filtered using filter paper, and distilled water was added to make up the volume to 50 ml. These prepared samples were stored under refrigerated conditions until analysis. Metal levels in different organs were determined using an Atomic Inductively Coupled Plasma Spectrophotometer (PG-900, ICEP-OES) [20].

Statistical Analysis

The collected data were analyzed using standard statistical methods, including the calculation of mean, standard error of the mean, and range. The statistical analysis was performed utilizing IBM SPSS (version 21). The significance of the observed differences was tested using an unpaired t-test at the 0.05 significance level. Additionally, Pearson correlation coefficients were calculated to assess the relationships between different variables [21].

RESULT AND DISCUSSION

Hematological Analysis

Hematological analysis revealed a White Blood Cell (WBC) concentration ranging from 150 to 400 (mean ± SE: 238 ± 6.128 x103/µL), while the concentration of Red Blood Cells (RBC) ranged from 4.5 to 6.0 (mean ± SE: 4.31 ± 0.045 x106 /µL). The Hemoglobin level ranged from 14 to 17 (mean ± SE: 23.0 ± 1.247 g/dl), and the blood parameter percentage was within the range of 38 to 58 (mean ± SE: 61.6 ± 1.122%).

Hematocrit percentage and Mean Corpuscular Volume (MCV) were found to range from 80 to 96 (mean ± SE: 141 ± 1.435 FL) and 27 to 32 (mean ± SE: 53.3 ± 1.717 pg), respectively. The Mean Corpuscular Hemoglobin (MCH) had a mean value of 27 to 32 (mean ± SE: 53.3 ± 1.717 pg), with the Mean Corpuscular Hemoglobin Concentration (MCHC) ranging from 30 to 35 g (mean ± SE: 36.7 ± 1.639 g/dl). The Platelet count was observed in the range of 4 to 10 (mean ± SE: 3.00 ± 0.816 x103/ µL), while Red Cell Distribution Width (RDW) ranged from 52.7 to 65.2 (mean ± SE: 59.6 ± 1.212 FL).

Furthermore, the differential white blood cell counts showed Neutrophils % in the range of 40 to 75 (mean ± SE: 81 ± 1.633%), Lymphocytes ranging from 20 to 45 (mean ± SE: 2 ± 0.816%), Monocytes in the range of 2 to 10 (mean ± SE: 2 ± 0.816%), and Eosinophils ranging from 1 to 6 (mean ± SE: 2 ± 0.816%) (Table 1).

Table 1: Hematological variables in Sindh sparrow poised from Swamplands of District Bahawalnagar

Variable (n = 20)	Mean ± SD	Range (Min-Max)
RBC($x10^6$/µL)	4.31±0.045	4.5-6
HGB(g/dl)	23.0±1.247	14-17
HCT(%)	61.6± 1.122	38-52
MCV(FL)	141.9± 1.435	80-96
MCH (pg)	53.3± 1.717	27-32
MCHC(g/dl)	37.6± 1.639	30-35
PLT($x10^3$/µL)	3± 0.816	4-10
RDW	59.6± 1.212	52.7-65.2
Neutrophils	81±1.633	40-75
Lymphocytes	15±2.494	20-45
Monocytes	2±0.816	2-10
Eosinophils	2±0.816	1-6

Heavy metals Concentration

The evaluation of Zinc (Zn), Chromium (Cr), Cadmium (Cd), Nickel (Ni), and Iron (Fe) levels was conducted in the muscle, liver, and kidney of Sindh sparrows. In the muscle, liver, and kidney, the levels of

Zinc were found to be 0.155±0.065 µg/kg, 0.11±0.09 µg/kg, and 0.525± 0.075 µg/kg, respectively, with highly significant differences observed between the organs. Chromium concentration in the muscle was 0.13 ± 0.05 µg/kg, while in the liver and kidney, it was 0.255 ± 0.075 kg/kg and 0.05± 0.03 µg/kg, respectively. The levels of Cadmium in the muscle, liver, and kidney were found to be 0.06 ± 0.03 µg/kg, 0.09 ± 0.03 µg/Kg, and 0.265 ± 0.065 µg/kg, respectively, with no substantial difference between the means of the organs. Nickel had mean values of 0.375 ± 0.075 µg/kg, 0.54 ± 0.05 µg/kg, and 0.275 ± 0.065 µg/kg in the muscle, liver, and kidney, respectively, with a non-significant mean difference. In contrast, Iron concentration in the muscle, liver, and kidney was 5.565 ± 0.645 mg/kg, 6.05 ± 0.85 mg/kg, and 3.54 ± 0.67 mg/kg, respectively, with highly significant differences in means.

The correlation matrix between the concentrations of the metals in the kidney indicated significant relationships for Pb, Ni, Cr, Zn, and Cd. The correlation matrix for metal concentrations in the liver revealed significant differences between all metals, indicating relationships among them. The detailed correlations between metals in the liver are compiled in Table 2, demonstrating significant relationships among all the assessed metals.

Table 2: Heavy metals concentration in liver, muscle and kidney of Sindh Sparrow

Metal	Organs	N	Range (Minimum-Maximum)	Mean ± Std. Deviation	SE	F-Value	P-Value
Zn	Muscle	20	0.09-0.22	0.155± 0.065	0.015	26	0.018
	Liver	20	0.02-0.20	0.11± 0.09	0.02		
	Kidney	20	0.450.60	0.525± 0.075	0.017		
Cr	Muscle	20	0.08-0.18	0.13± 0.05	0.011	10.65	0.011
	Liver	20	0.18-0.33	0.255± 0.075	0.017		
	Kidney	20	0.02-0.08	0.05± 0.03	0.007		
Cd	Muscle	20	0.03-0.09	0.06± 0.03	0.007	14.66	0.005
	Liver	20	0.03-0.15	0.09±0.06	0.013		
	Kidney	20	0.21-0.32	0.265± 0.055	0.012		
Ni	Muscle	20	0.30-0.45	0.375± 0.075	0.017	13.05	0.007
	Liver	20	0.49-0.59	0.54± 0.05	0.011		
	Kidney	20	0.21-0.34	0.275± 0.065	0.015		
Fe	Muscle	20	4.92-6.21	5.565± 0.645	0.144	10.05	0.012
	Liver	20	5.20-6.90	6.05± 0.85	0.19		
	Kidney	20	2.87-4.21	3.54± 0.67	0.15		

SE = Standard error NS = Non-significant (P>0.05): Highly significant (P<0.01) * = Significant (P<0.05)

Correlation matrix of different metals in the body organs

In the muscle of Sindh Sparrows, significant correlations were observed, including a highly negative correlation between Chromium (Cr) and Zinc (Zn), and positive correlations of Cadmium (Cd), Nickel (Ni), and Iron (Fe) with Zn. Additionally, Cd, Ni, and Fe showed positive correlations with Cr, while Ni, Fe, and Cd exhibited positive correlations with each other. These correlations were found to be statistically significant ($p < 0.05$). Similarly, in the Pearson Correlation analysis, significant positive correlations were noted between Cr and Zn, as well as positive correlations of Cd, Ni, and Fe with both Zn and Cr. Noteworthy was the positive correlation between Ni and Cd, and Ni and Fe, with a negative correlation observed between Fe and Cd. The correlation between Cd and Cr was significant, and all other correlations reached statistical significance ($p < 0.05$). The correlation matrix for heavy metals in the kidney of Sindh Sparrows revealed significant correlations of Cr, Ni, and Fe with Zn, positive correlations of Cd and Fe with Cr, and positive correlations among Cd, Ni, and Fe. All these correlations were found to be statistically significant ($p < 0.05$). These findings suggest intricate relationships in the accumulation patterns of heavy metals in different organs of the Sindh Sparrows, indicating potential shared pathways and interactions among these elements. This is shown in table 3.

Table 3: The correlation matrix of heavy metals in muscles, liver and kidneys in Sindh Sparrow

	Pearson Correlation (2-tailed)		Zn	Cr	Cd	Ni	Fe
Muscles	Cr	Pearson Correlation	0.954				
		Sig.(2-tailed)	0.194				

		N	20				
	Cd	Pearson Correlation	0.999	0.963			
		Sig.(2-tailed)	0.021	0.173			
		N	20	20			
	Ni	Pearson Correlation	0.974	0.997	0.981		
		Sig.(2-tailed)	0.146	0.048	0.126		
		N	20	20	20		
	Fe	Pearson Correlation	0.998	0.972	0.999	0.987	
		Sig.(2-tailed)	0.044	0.151	0.023	0.103	
		N	20	20	20	20	
	Pearson Correlation (2-tailed)		**Zn**	**Cr**	**Cd**	**Ni**	**Fe**
Liver	Cr	Pearson Correlation	0.998				
		Sig.(2-tailed)	0.036				
		N	20				
	Cd	Pearson Correlation	0.996	0.99			
		Sig.(2-tailed)	0.056	0.091			
		N	20	20			
	Ni	Pearson Correlation	0.99	0.997	0.975		
		Sig.(2-tailed)	0.088	0.052	0.144		
		N	20	20	20		
	Fe	Pearson Correlation	0.995	0.999	0.983	0.999	
		Sig.(2-tailed)	0.063	0.028	0.119	0.024	
		N	20	20	20	20	
	Pearson Correlation (2-tailed)		**Zn**	**Cr**	**Cd**	**Ni**	**Fe**
Kidney	Cr	Pearson Correlation	0.998				
		Sig.(2-tailed)	0.036				
		N	20				
	Cd	Pearson Correlation	0.996	0.99			
		Sig.(2-tailed)	0.056	0.091			
		N	20	20			
	Ni	Pearson Correlation	0.99	0.997	0.975		
		Sig.(2-tailed)	0.088	0.052	0.144		
		N	20	20	20		
	Fe	Pearson Correlation	0.995	0.999	0.983	0.999	
		Sig.(2-tailed)	0.063	0.028	0.119	0.024	
		N	20	20	20	20	

Upper values indicated Pearson's correlation coefficient; Lower values indicated level of significance at 5% probability. Significance (P<0.05); -Highly significant (P<0.01)

Heavy metals comparison in the body organs

Significant correlations were observed among the concentrations of Zinc (Zn), Chromium (Cr), Cadmium (Cd), Nickel (Ni), and Iron (Fe) in the kidney, liver, and muscles of both male and female Sindh sparrows. In terms of Zn, its concentration was found to be higher in the kidney than in the liver and muscles, following the pattern Kidney > Muscles > Liver. Conversely, Chromium (Cr) exhibited higher concentrations in the liver compared to other organs, aligning with the order Liver > Muscles > Kidney. Cadmium (Cd) concentrations were notably elevated in the kidneys, displaying the pattern Kidneys > Liver > Muscles. Nickel (Ni) concentrations were found to be higher in the liver, following the order Liver > Muscles > Kidneys. The concentration of Iron (Fe) displayed higher levels in the liver and lower levels in the kidneys, indicating the pattern Liver > Muscles > Kidneys. These patterns suggest distinct accumulation tendencies of these heavy metals in different organs of male and female Sindh sparrows.

Discussion

The objective of our study was to analyze hematological parameters and assess heavy metal concentrations in the muscle, liver, and kidney of the Sindh sparrow. The hemoglobin concentration in Sindh sparrows ranged

from 22.75 to 25.9 g/dl, slightly higher than a previous finding of 17.541 ± 4 0.80 g/dl. Interestingly, we observed a slightly positive correlation between body length and hemoglobin (r = 0.15), contrary to Minias' findings of a negative correlation between body size and hemoglobin. Additionally, lymphocytes were highly negatively correlated with body weight (r = -0.80) in Sindh Sparrow, contrasting with a positive correlation reported in another study. Our investigation also discussed various blood parameters, such as HGB, WBCs, Total RBCs, HCT/PCV, MOV, MCH, MCHC, Platelets, RDW, MPV, Neutrophils, Lymphocytes, Monocytes, and Eosinocytes in Sindh Sparrow, providing novel insights [22].

Regarding heavy metal concentrations, our study found a zinc concentration in the liver of Sindh sparrow to be 0.11 ± 0.09 µg/kg, consistent with a previous study [23] that reported 94 µg/kg of Zn. In contrast, another study [24] found a Zn concentration of 0.22 ± 0.065 µg/kg from feathers of the passerine family. Notably, the concentration of Fe was found to be the highest in all three organs (muscle, liver, and kidney), aligning with analogous findings in feathers of Sindh Sparrows and other bird species [24] Lead and chromium, known toxic metals, accumulate in the skeleton, as demonstrated by a study on Larus argentatus. In our study, zinc and iron concentrations showed significant differences, while chromium, cadmium, and nickel showed non-significant differences. Other studies [25]. have highlighted the elevated lead concentrations in urban bird populations compared to those in agricultural habitats, emphasizing the impact of diet on the specific accumulation patterns of toxic elements [26-28].

Conclusion

In conclusion, the hematological analysis of sparrows exhibited variability within the studied range. The concentrations of different heavy metals demonstrated fluctuations, with significant differences noted in the concentrations of zinc and iron, while chromium, cadmium, and nickel exhibited non-significant differences. The patterns of heavy metal accumulation in various organs revealed higher concentrations of chromium, nickel, and iron in the liver, while cadmium and zinc were more concentrated in the kidneys. Additionally, significant correlations were observed among the concentrations of Zinc (Zn), Chromium (Cr), Cadmium (Cd), Nickel (Ni), and Iron (Fe) in the kidney, liver, and muscles of both male and female Sindh sparrows. Further studies are warranted to comprehensively assess the accumulation levels of heavy metals in birds, emphasizing the urgency to address environmental pollution that poses risks to both avian species and human populations worldwide. Controlling environmental pollution is imperative for the well-being of birds and the broader ecosystem.

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