Heavy Metal Residues In Liver Tissues Of Selected Birds From Aquatic And Terrestrial Environments Of Visakhapatnam, India.

Sanchari Biswas, Ch Ramakrishna, Y A Maruthi

Abstract: Visakhapatnam is a fast growing city with a rich diversity of flora and fauna. In the recent years, the avifauna of Visakhapatnam has faced intensive number of increasing threats due to various anthropogenic activities out of which heavy metal contamination is one. The present study was carried out to determine the concentration of heavy metals Pb, As, Ni, Mn, Cu, Co in Liver tissues of five bird species namely White Breasted Water hen (Amaurornis phoenicurus), Black Kite (Milvus migrans), Northern Pintail (Anas acuta), White Throated Kingfisher(Halcyon smyrnensis) and Asian Koel (Eudynamys scolopaceus) from various aquatic and terrestrial ecosystems. Heavy metal residues were detected using ICPMS and statistical analysis (using Origin Lab 2019) were applied to validate the results. The heavy metal accumulation in the liver tissue samples of the birds followed the order Cu>Mn>As>Pb>Ni>Co. With the application of statistical analysis, significant strong positive correlations were obtained between various species of birds and their metal accumulating pattern with Cu and Mn being the principle two elements absorbed. The overall results obtained in the study suggests the silent ongoing intake of heavy metals by living forms through their food chain which may give rise to biomagnification with time.

Keywords: Anthropogenic activity, birds, Biomagnification, ecosystems, heavy metals, environmental contaminants, tissues.

1. INTRODUCTION

IN recent years, humans are vulnerable to the detrimental effects of environmental contamination arising from the increased rate of different agricultural production, industrial activities and commercial mining operations. Because of certain anthropogenic activities like discharges from mines, industrial activities, hazardous industrial wastes disposal, burning of fossil fuels, paints and fertilizers, manures, open incineration [43];[44], there is increasing levels of heavy metals in the environment [28]. Grúz et al., [17] stated that the accumulation of heavy metals can be easily detected and monitored in human or livestock, whereas it is difficult and nearly impossible to regulate and check the same in the diet of wild animals. Birds are more susceptible to the ongoing environmental contamination as they occupy a higher position in the trophic level of food chains [24]. Several studies have been carried out on heavy metals that indicates how heavy metals accumulate in bones, blood, egg, feathers, kidney, liver, blood and excreta [17]; [38]; [16]; [7]; [5]; [27]; [4]; [19]; [8]; [6]; [29]; [11]. Due to their longevity and being on higher trophic level within an ecosystem, predatory birds are sensitive to insignificant changes in their surrounding environment. Long term exposure of birds to toxic metals at inadequate levels lead to mortality and may give rise to other health issues such as hindered procreation, susceptibility to anydisease, stress [15] and behavioral changes. Hence, examining the extent of heavy metal contamination from a particular area is convenient for not only determining the health of a species but also in studying variations in levels of contaminant in the environment niche of a species [10];[19].In Indian context, studies carried out on bioaccumulation on birds are limited but some of them have reflected significantly higher levels of metals [32]; [26]; [21]; [41]; [31]; [27]; [7]; [16]; [38].

Visakhapatnam, Andhra Pradesh, India is a fast growing city with rich avifauna but since the past few years both aquatic and terrestrial ecosystems in the region have been facing the consequences of anthropogenic threats like urbanization. Because of limited studies in the region, it is important to establish elemental characterization data to understand the impacts of the organic pollutants on birds. In Southern India, researchers have carried out bioaccumulation studies on few terrestrial and water birds in Nilgiris, Karnataka and Tiruchirapalli, Tamil Nadu. However, studies on the same from Visakhapatnam, Andhra Pradesh remain limited. Therefore, the main objective of our study was to investigate concentrations of heavy metal in birds from aquatic and terrestrial ecosystems. Additionally, literature suggests that birds are easy to sample from dead, alive or museum specimens including endangered species [27].

2. MATERIALS AND METHODS

As per the bioethical concerns, no live birds were harmed during our study. Opportunistic collection of dead and moribund birds were carried out from various aquatic and terrestrial ecosystems. The methodology has been described in four different sections which are as follows.

2.1 Study Area

Visakhapatnam has a geographical area of 682 km^2. It is situated in the vicinity of Eastern Ghats which hosts a diversity of flora and fauna among the hills along with a few naturally formed and manmade reservoirs. Our study area and sampling points consisted of terrestrial and aquatic ecosystems depending on the type of specimens procured opportunistically. A total of 42 specimens including sub adults and juveniles were collected from Meghadrigedda, Simhachalam, Kailasagiri and Rushikonda.

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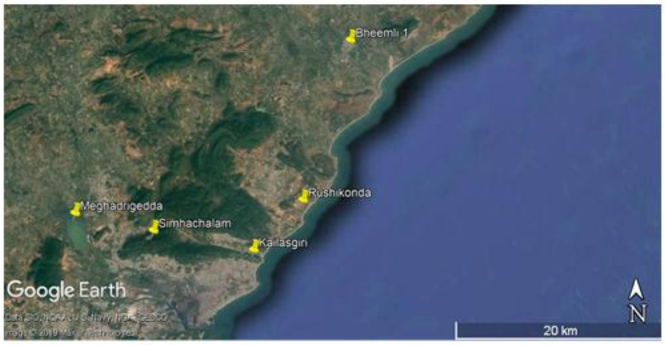

Fig. 1. Map Showing Study Area and sampling locations (Source: Google Earth)

2.2 Necropsy of Samples
Considering bioethical norms, only dead birds collected opportunistically were used in the study. Necropsy of each specimens were carried out individually. The procedure was initiated by making lines of incision with help of scalpel at the posterior sides of the abdomen and then cutting the breast tissue open. Observations were made in order to find exudates, fluids and blood clots. After removal of the visceral contents, the liver tissue was removed and wrapped in aluminum foil and stored in deep freezer (-20 degree Celsius) for further analysis.

2.3 Acid Digestion Procedure
The liver tissues were oven dried using Hot Air Oven at 80 degrees Celsius for 24 hours. Following standard technique suggested by [20], 1-5 g of each sample was weighed and transferred into Teflon beakers followed by addition of 10 mL HNO_3 (69% Emplura Merck) and heated on Hot Plate for about 30 mins followed by addition of $HClO_4$ (60% Emparta ACS) and reheated for 15 mins until a thick transparent solution was obtained. Following this, H_2O_2 (30% Emplura Merck) was finally added and heated for 10 mins in order to digest excessive organic matter present in the samples.All digested samples were finally filtered using Whatman No. 1 filter paper and volume was made up to 25 mL with Millipore water and stored in pre-cleaned polythene vials and refrigerated for analysis.

2.4 Elemental Analysis
Heavy Metals namely Mn, Ni, Pb, As, Cu and Co were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICPMS), Agilent 770s, Agilent Technologies, Japan. Standards for all the elements manufactured by NIST (National Institute of Standard Technology), U.S were utilized for calibration. The detection limit of each standard were 0.5 ppb, 1 ppb, 5 ppb, 10 ppb, 25 ppb, 50 ppb, 100 ppb respectively. The results obtained were expressed in ppm (ug/g) where a conversion factor of three was used in order to convert ppb to ppm.

3 RESULTS
A total of 42 specimens were collected from five different regions of Visakhapatnam which is represented in Table 2. The concentrations of heavy metals Mn, Cu, Co, Pb, Ni and As were determined in this study and has been expressed in dry weight as dry weight values are more compatible and authentic compared to wet weight values [1]; [37]; [38]. Metal concentrations were detected among various species of birds. Comparatively, the concentration of Manganese was detected to be highest (2.25 ug/g) in liver tissue of White Breasted Water Hen. Whereas considering the mean of all samples, in comparison to Ni, Pb, As, Co and Mn, Cu (Copper) was detected highest (o.88 ug/g) followed by Mn which was 0.67 ug/g followed by As (0.09 ug/g) and Pb (0.06 ug/g). On an average, the liver tissue of White Breasted Water Hen had the highest concentration of metals followed by Black Kite>Northern Pintail>Asian Koel> White Throated Kingfisher.

TABLE 1

CONCENTRATIONS OF HEAVY METALS IN FIVE SPECIES OF BIRDS

Metal (ug/g)	White Breasted Water Hen	Black Kite	Northern Pintail	White Throated Kingfisher	Asian Koel
Mn	2.258981	0.377491	0.135963	0.107823	0.501089
Ni	0.08861	0.080839	0.026621	0.033618	0.070918
Cu	0.310389	1.897414	1.035353	0.695228	0.4735
Co	0.007128	0.00509	0.001379	0.001645	0.008664
Pb	0.110468	0.039457	0.071924	0.039649	0.051331
As	0.012269	0.344414	0.005655	0.005788	0.120492

TABLE 2

BIRD STATISTICS AND SAMPLING LOCATIONS

S. No	Common Name	Scientific Name	Sampling Area	Sample Size	Population Trend, IUCN 2019
1	White Breasted Water Hen	Amaurornis phoenicurus	Rushikonda	8	unknown
2	Black Kite	Milvus migrans	Kailasgiri	10	Stable
3	Northern Pintail	Anas acuta	Meghadrigeda	8	Decreasing
4	White Throated Kingfisher	Halcyon smyrnensis	Bheemli		unknown
5	Asian Koel	Eudynamys scolopaceus	Simhachalam	16	unknown

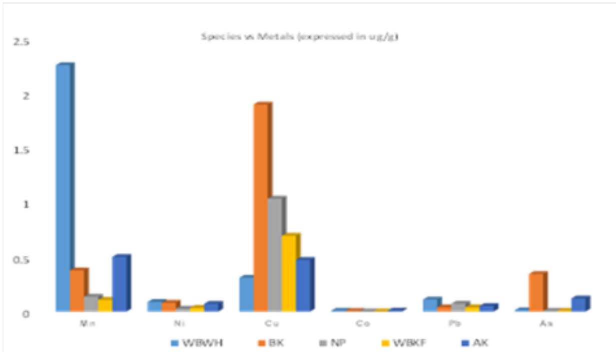

Fig. 2. Heavy metal (Mn,Cu,Pb,Ni,As and Co) concentration in five different species of birds (Table 3) (Results are expressed in ug/g)

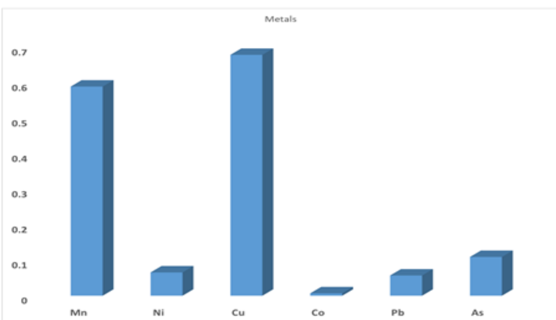

Fig. 3. Mean concentrations of Heavy metals (Mn,Cu,Pb,Ni,As and Co) expressed in ug/g)

7 DISCUSSION

7.1 Copper

On an average, Copper (Cu) content was highest (0.88 ug/g) among all the metals studied. This was followed by Mn (0.67) ug/g and As (0.09) ug/g. The average concentration of Pb was 0.06 ug/g. The liver tissues of Black Kite (Milvus migrans) had Cu content (1.89) ug/g whereas in Northern Pintail (Anas acuta) it was 1.03 ug/g. A similar study done by [27], at Tiruchirapalli revealed 53.13 ug/g of Cu in Black Kite feathers and 139.01 ug/g in feathers of Cattle Egret (Egreta garzetta). [24] found 73.58 ug/g Copper (Cu) in liver tissue of Bar-headed Goose whereas lesser concentration of Cu (2.95 ug/g) was found in Eurasian Collared Dove. Previous studies done by [19] revealed 4.12 ug/g of Cu in liver tissues of Common Myna and 2.78 ug/g Cu in Jungle Babbler. Compared to the studies done in Sargodha district, Punjab [33] where 0.21±0.02 ug/g Cu content was found in liver of House sparrow (Passer domesticus) and 0.29 ± 0.03 in Black Kite (Milvus migrans) liver tissue, the present study revealed higher Cu content in liver tissues of Black Kite (1.89) ug/g and 1.03 ug/g in Northern Pintail (Anas acuta). Antifouling paints are the major culprits of copper discharge causing environmental contamination and waterfowls are often affected by ingestion of the intoxicated contents [14]. Other sources of Copper mobilisation in the environment include agricultural and waste disposal, ore extraction like mining and smelting. Deposition of Cu in soil occurs through application of insecticides and fungicides [42]; [9]. [42] stated that Cattle egrets are susceptible to Cu intoxication due to spraying of fungicides and Cu bioavailability in the environment. However, birds tend to tolerate higher levels of Copper in their system compared to mammals [36]; [24]. Frank and Borg (1979) [13] reported Copper residues in liver of Mute Swans upto to 1000 μg/g. Therefore, in the present study, the concentrations of Cu reported in all five species of birds were less than Cu levels capable of impelling toxicity.

TABLE 3
ABBREVIATIONS USED FOR BIRDS SAMPLES

S.No	Common Name	Sample Code
1	White Breasted Water Hen	WBWH
2	Black Kite	BK
3	Northern Pintail	NP
4	White Throated Kingfisher	WBKF
5	Asian Koel	AK

7.2 Manganese

On an average, the content of Manganese (Mn) in the samples detected was (0.67) ug/g out of which White Breasted Water Hen (Amaurornis phoenicurus) had the highest concentration (2.25 ± 0.89) ug/g in liver tissues. Previously, as studied by [39] in the same region among different species, House Crow (Corvus splendens) liver tissues reported higher levels of Mn (17.4±0.50) ug/g which was close to the levels reported by [27] in House Crow (22.82) ug/g and Black Kite (14.81) ug/g respectively. [41] reported high levels of Mn in excreta (45.58 – 212.3) ug/g of Black Kite (Milvus migrans). However, the content of Mn reported in our study are in agreement with levels found in Black Crowned Night Heron [25] (2.25) ug/g and also with liver tissues of Black Kite (2.19 ± 0.13) ug/g conducted in Sargodha district, Punjab [42]
Igneous rocks contain Mn and it is also one of the essential micronutrient. The mobilization of Mn into the environment is through natural sources like ocean spray, weathering of rocks and vulcanization [42] and can easily cause food contamination flowing through food chain [31]; [27]. Other anthropogenic sources of Mn in environment include urban waste dumps, industrial effluents [31]; [27] incineration of waste in catchment areas, major traffic activities, incomplete combustion of fuel and leaded petrol contribute to increased atmospheric concentration of Mn [45]; [42]. [18] concluded that Mn ingested through contaminated food by birds are also one of the major sources of Mn in feathers. When compared, the concentration of Mn in our samples were much lower than Mn content in feathers of cattle egrets in other regions [8]; [23]. However, concentrations of Mn reported in the present study from India was comparatively higher than Wandering Albatross (1.00 ug/g); Grey-Headed Albatross (1.291) ug/g

7.3 Arsenic

The average concentration of Arsenic (As) in the present study was 0.09 ug/g among other samples out of which Black Kite (Milvus migrans) liver tissue had the highest concentration (0.09 ug/g). However, previous study by [38] in the same region reported higher concentration (1.87 ± 0.78) ug/g in tissues of House Crow (Corvus splendens). [41] reported comparatively higher concentration of Arsenic in excreta of similar species in the range (0.78 ± 2.14) ug/g from Ludhiana, Punjab. Arsenic (As) is a threat to wildlife in estuarine and marine ecosystems [34]. Anthropogenic sources of Arsenic into the environment are due to mining and smelting activities by refining ores of heavy metals, combustion of fossil fuels, industrialization, agricultural and pesticide runoff and Mn has been extensively used to promote livestock growth etc [3];[30]. The concentration of As in the present study was found to be much lesser than the values (0.15 - 1.6) ug/g as reported by [25]. The concentration of Pb and Co in the current study are comparatively lesser than the values of Pb (0.37–15.44) ug/g as reported by Kirubhanandhini et al., 2019 in different species of birds. The average Cobalt (Co) levels in the current study are less compared to other studies reported from Pakistan (0.52) ug/g by [33] ug/g, northern giant petrel (0.608 ug/g), diving petrel (0.954 ug/g) and southern giant petrel (0.587 ug/g); [42].

3.2 Statistical Analysis

On applying Pearson's Correlation Coefficient (r) for concentration of metals in liver tissues of birds, significant positive correlations (Table 5) were obtained between White Breasted Water Hen and Asian Koel (r=0.73), Black Kite and Northern Pintail (r=0.97), Black Kite and White Throated Kingfisher (r=0.98), Northern Pintail and White Throated Kingfisher (r=0.99), where P<0.05 which suggests similar metal accumulation pattern in liver of both water birds. Using Origin Lab 2019 A PCA was conducted to infer the hypothetical sources of heavy metals (natural or anthropogenic) following the standard procedure reported in the literature, [12]; [22] which showed clustering of the variables into different groups, where variables belonging to one group are highly correlated with each other [2]. The PCA was performed on the dimensionless standardized form of the data set and is presented in Figure 2. The Varimax rotation was used to maximize the sum of the variance of the factor coefficients. In the PCA analysis, 5 PCAs were computed, and the variances explained by them were 71.99%, 99.27%, 99.97%,99.99% and 100 % for birds (table 5), respectively (Figure 5). Two Groups (WBWH, AK) consists Mn as a major element and remaining three groups (BK, NP, WBKF) consists Cu as a major element. (figure 4)

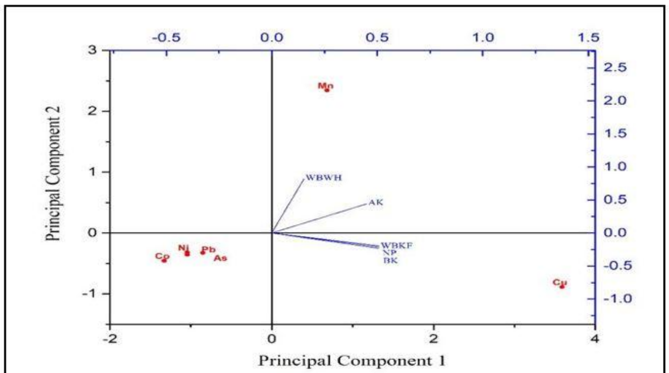

Fig. 4 PCA of Heavy metals in Bird Samples

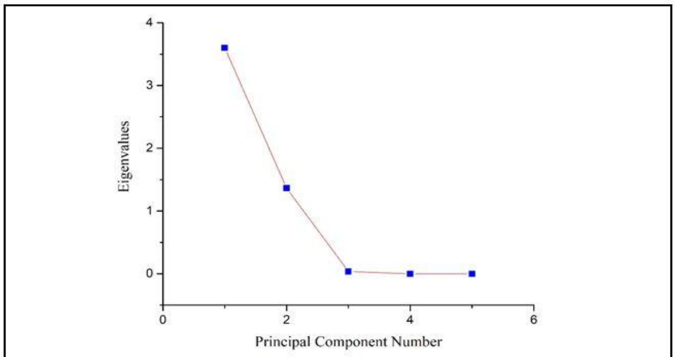

Fig. 5 Factor analysis of elements in bird samples

TABLE 4

CORRELATION AMONG THE SPECIES IN PRESENT STUDY

	WBWH	BK	NP	WBKF	AK
WBWH	1				
BK	0.056878	1			
NP	0.025341	0.979915	1		
WBKF	0.046776	0.981332	0.999361	1	
AK	0.734069	0.716865	0.672947	0.689528	1

TABLE 5

EXPLANATION FOR VARIANCE OF ELEMENTS IN BIRD SAMPLE

COMPONENTS	INITIAL EIGENVALUES	
	% Variance	% Cumulative
1	71.99	71.99
2	27.27	99.27
3	0.71	99.97
4	0.02	99.99
5	0.01	100

The present study reported Mn and Cu being the principle two elements being accumulated in the tissues of birds. Several other studies have reported similar pattern of accumulation of Mn and Cu in other life forms. [35] reported that Mn and Cu accumulation in female fish gonads was more significant when compared to the case of Zn and Fe. In another study by [40] on influence of Manganese exposure on the distribution of essential trace elements conducted on rat brain, similar positive correlation between Mn and Cu was reported.

3.2.1 Kruskal-Wallis ANOVA

The Kruskal-Wallis test is a nonparametric test which is used for determining whether three or more independent samples originate from the same population. When this test leads to significant results, at least one sample differs from the others. The p value is 0.7 which confirms that groups are not significantly different.

Input Data

	Data	Range
Metals	[Book1]Sheet1!A"Metals"	[1*:6*]
WBWH	[Book1]Sheet1!B"WBWH"	[1*:6*]
BK	[Book1]Sheet1!C"BK"	[1*:6*]
NP	[Book1]Sheet1!D"NP"	[1*:6*]
WBKF	[Book1]Sheet1!E"WBKF"	[1*:6*]
AK	[Book1]Sheet1!F"AK"	[1*:6*]

Descriptive Statistics

	N	Min	Q1	Median	Q3	Max
"Metals"	6	0.00713	0.01098	0.09954	0.79754	2.25898
"WBWH"	6	0.00509	0.03087	0.21263	0.75747	1.89741
"BK"	6	0.00138	0.00459	0.04927	0.36081	1.03535
"NP"	6	0.00165	0.00475	0.03663	0.25467	0.69523
"WBKF"	6	0.00866	0.04066	0.0957	0.4804	0.50109

Ranks

	N	Mean Rank	Sum Rank
"Metals"	6	17	102
"WBWH"	6	17.66667	106
"BK"	6	13	78
"NP"	6	12.33333	74
"WBKF"	6	17.5	105

Test Statistics

	Chi-Square	DF	Prob>Chi-Square
	2.10753	4	0.71599

Fig 6: *Kruskal-Walis ANOVA test*

4 CONCLUSION

Visakhapatnam is a smart city which is rapidly growing everyday due to urbanisation alongside with its consequences on nature. [4]. The city owns a number of small and large scale industries including Visakhapatnam Port, Steel Plant, tanneries, and Petroleum Industry. [38]. The results obtained in the present study suggests that Copper content found in the tissues of all birds might be due to anthropogenic sources ranging from paint industries, waste disposal and other major industrial non-point sources polluting both terrestrial and aquatic environments. The presence of Mn in all samples might be because of natural processes like weathering of rocks and Ocean spray and manmade sources like industrial effluents, waste disposal etc. Irrespective of control measures, the entry of heavy metals into our environment is inevitable and it continues to multiply through each trophic level leading to biomagnification. Humans being on top of the food chain are the most susceptible to the same. However, the levels of metals found in the present study were below levels that are associated with toxicological effects.

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