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Feathers of *Bulbulcus ibis* (L.) as a non-destructive biomonitoring tool for assessment of lead pollution: A case study from various severely contaminated wetland habitats

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ABSTRACT

Present study aims to determine the lead (Pb) concentrations in the feather samples of cattle egret (*Bulbulcus ibis*) found in different contaminated wetland habitats present in the state Odisha, India. This study also focused on the heavy metal concentrations of top layer soil and on the level of metal accumulation in prey samples (i.e. small fishes) preferred by cattle egrets for explaining the level of amplification of Pb in different polluted wetland habitats. Moreover, reasons behind the variations of Pb concentrations within the sampled wetland habitats are explained. Feathers, Prey samples and sediments from their foraging habitats were collected from eight different heronries. Determination of Pb concentrations in samples has undergone acid digestion followed by elemental analysis (i.e. Pb) in Atomic Absorption Spectrophotometer (AAS). The results indicated that Pb concentration was varied significantly in soil (15.13-56.26µg/g), Prey (0.21-55.76µg/g) and feathers (5.6-49.86µg/g) in different sites. The highest concentration was found at Hirakud in soil and prey. However, feather samples showed the highest concentrations of lead in Talcher may be due to the mining activities in and around the sampling location. Present study found the positive correlation ($p<0.05$) between soil, prey and feather samples of cattle egret among the eight sampling locations may be due to many of the mining, industrial and anthropogenic sources. The processes like geophagy and biomagnification may contribute to the positive correlation among the sampling habitats. Concluding this work, it might be confirmed that avian feathers can be used as non-destructive biomonitoring tool for assessment of the heavy metal contamination level in various segments of different ecosystems. Hence, based on the results of the study, the environmental management plan could be suggested and implemented for better conservation of the avian fauna.

Keywords:*Cattle egret; Feather; Lead; Bioaccumulation; Heavy metals.*

1. INTRODUCTION

Urbanization and industrial processes are continuously introducing pollutants to the ecosystem. The geological cycle, volcanic eruption and different anthropogenic sources are also contributing heavy metals to the environment. Especially the heavy metal contamination is a global concern due to its bio accumulation potential. The ubiquitous and non- biodegradable nature of heavy metal affects the structural and functional integrity of ecosystem [1]. Many of the biological organisms used as indicators for explaining contamination level of an ecosystem [2–5]. Pb is the most toxic metal among the all other heavy metals present in the environment and the bioaccumulation potential of this heavy metal in different trophic level ultimately affect human health [6,7]. Pb causes hematological toxicity and primarily accumulates in bones and kidneys of Cattle egret female in major concentration than male ones [8]. This metal also is known to cause thermoregulation impairment and produces adverse reproductive effect resulting in decreased survival nestlings of birds [9,10]. Injection of food and water are the general processes for the uptake of heavy metal in birds. Birds are also exposed to heavy metal by the process of geophagy, which is the eating practice of soil like substances for getting essential nutrients from soil. Egrets of the family-Ardeidae are wading water birds that feed on diverse aquatic and wetland habitat. Generally, Cattle egrets are feeding on a wide range of vertebrates and invertebrates [6]. Contaminated soils are the major heavy metal sources and through food it is getting accumulated in egret species [11,12]. This study aims to determine the Pb concentration in the feather of cattle egrets, in the prey samples of cattle egrets and surroundings surface soil of contaminated wetland habitats of egret heronries. The study describes Pb contamination in the feather sample of Cattle egrets from different wetland habitats present in Odisha, Eastern India, which has been further justified by using statistical analysis. This study can be used as a tool for biomonitoring of heavy metal pollution in contaminated habitats and also for eco-toxicological evaluation of the cattle egret species.

2. MATERIALS AND METHODS

2.1. Study area.

Eight different heronries are sampled from different districts of Odisha for their different exposure to contamination, presence of breeding egret colonies and sampling suitability has shown in Figure 1. The 1st sampling site Mangalajodi area (19°53'16.78"N, 85°26'19.55"E) of Chilika is a large bird congregation site all over the year for migratory as well as resident birds in Khurda district. The 2nd sampling site Hirakud reservoir

is one of the largest dams as well as the largest reservoir of India. The south western part of the reservoir near Debrigarh Wildlife Sanctuary (21°28'56.03"N, 83°48'53.73"E) of Bargarh district is sampled. The 3rd sampling site is a place named Tihidi in Bhadrak district of Odisha.

Figure 1. Map of study area showing location of sampling sites.

Heronries in site 3 (20°59'30.4"N, 86°38'11.24"E) are surrounded by a large cultivation area. The 4th sampling site Chandaneswar of Balasore district is the northeast border of Odisha with West Bengal. Those heronries (21°38'1.03"N, 87°28'7.29"E) are in coastal region around 3-4 kilometers from the main tourist area. Talcher, the 5th sampling site (20°57'43.74"N, 85°13'20.03"E), is situated in Angul district. It is a highly urbanizing city with heavy industrial effects and mining activity. The 6th sampling site (19°53'34.36"N, 84°6'47.91"E) is Daringbadi. The sampled heronries are inside the forest patches near the outskirts of town. Titlagarh city of Bolangir district is the 7th sampling site (20°16'18.96"N, 83°9'10.26"E). It is a semi urban place which is known for its high temperature (avg-27.3°c and max- 48.5°c) in Odisha. The heronries are present at peripheral of the city and surrounded by many abandoned water bodies. The 8th sampling site (18°48'16.2"N, 82°42'41.17"E) is in Koraput district. The sampled heronries are near the town of Koraput with high anthropologic pressure like cattle and human interference.

2.2. Sample collection.

Feather, Prey and Soil sample collection and processing:
At each sampling site we have marked the sample nests of the egret colony. Priority was given to cattle egrets. Feather samples (n=3samples×8stations×3seasons×2year=144samples) were collected randomly from nests of cattle egrets and cleaned with 10% Nitric acid (HNO_3) and deionised water. After washing, feathers were oven dried at 110°c and then ground in mortar pastle. Prey samples of cattle egrets described above were collected (n=3samples×8stations×3seasons×2year=144samples) from the nests as well as from the foraging ground by targeting cattle egrets. Sometimes regurgitated food samples were also collected. The samples were washed with deionised water and 10% of HNO_3 to remove soil particles. Then the samples were dried and ground to form a powder in laboratory. Soil samples were collected (n=3samples×8stations×3seasons× 2year=144samples) from 5-10 cm depth from the foraging areas. Soil samples were dried and grained to pass through 2mm mesh sieve to obtain a fine powder.

2.3. Chemical analysis and Statistics.

From each sample 0.25gm of feather, prey sample and 0.5gm of soil sample were taken and transferred to digester tube for acid digestion in a microwave accelerated reaction system. Feather samples were digested according to Jones and Laslett [13] using ultrapure HNO_3. Prey and soil samples were digested with HNO_3 and Hydrochloric acid (HCl) in 1:3 ratio. Each acid digestion is compared by digestion blank. 25ml of feather and prey sample and 50ml of soil sample prepared using deionised water. The concentration of Pb was determined by Atomic Absorption Spectrophotometer of model Shimadzu, AA 6300 with wave length 217.0 nm, lamp current 5.0 mA (mili ampere). For Pb determination of every sample, mean concentration was taken. The results were compiled to form a database using MS Excel. Basic descriptive statistical analysis were done using RStudio 3.4.4 software. Data are expressed as mean ± SD. The minimum to maximum range was also described. One way analysis of variance (ANOVA) was used for site-specific differences based on mean concentration of Pb to determine whether the data are statistically significant or not. Difference and relation among concentration in feathers, prey and soil samples were tested using Spearmann's correlation plot.

3. RESULTS

3.1. Pb concentration in feather.

Basic descriptive statistics of Pb in feather of cattle egret are listed in Table 1. This table shows the amount of Pb concentration found from different parts of the study area. The highest concentration of Pb was found at Talcher with 44.94µg/g followed by Bhadrak with 36.59µg/g. Hirakud and Chandaneswar sites have found 30.55µg/g and 25.94µg/g respectively. Chilika and Koraput sites have nearly equal with 22µg/g of Pb. The lowest amount of metal detected at Daringbadi site with 8.19µg/g followed by Titlagarh with 8.75µg/g. The pattern of metal contamination in feathers among all the eight sites across the study area shows differences in Pb accumulation in the following order: Talcher>Bhadrak>Hirakud>Chandaneswar>Koraput>Chilika>Titlagarh>Daringbadi (Figure 2).

3.2. Pb concentration in prey sample.

Basic descriptive statistics of Pb from the prey samples of cattle egret are listed in Table 1. Among all the prey samples, Pb concentration is found highest at Hirakud with 48.88µg/g and lowest at Daringbadi with 2.04µg/g. Talcher, Bhadrak and Chandaneswar are the places where the accumulation is also much higher than Chilika, Daringbadi, Titlagarh and Koraput. The pattern of metal contamination among prey samples of all sites as

described in Box-plot (Figure 3) are in order of Hirakud>Talcher>Bhadrak>Chandaneswar>Koraput>Chilika>Titlagarh>Daringbadi.

3.3. Pb concentration in soil.

Basic descriptive statistics of Pb concentration in soil are shown in Table 1. Maximum concentration of Pb was found at the heronry of Hirakud with 52.46µg/g and minimum at Koraput. Bhadrak, Talcher and Titlagarh show the nearly equal presence of Pb 33.61µg/g, 31.09µg/g and 37.06µg/g respectively. Chandaneswar and Daringbadi have 25.78µg/g and 16.11µg/g respectively. The mean concentration of Pb in soil of all heronries as described in Box-plot (Figure 4) is in the order of Hirakud>Chilika>Titlagarh>Bhadrak>Talcher>Chandaneswar> Daringbadi>Koraput.

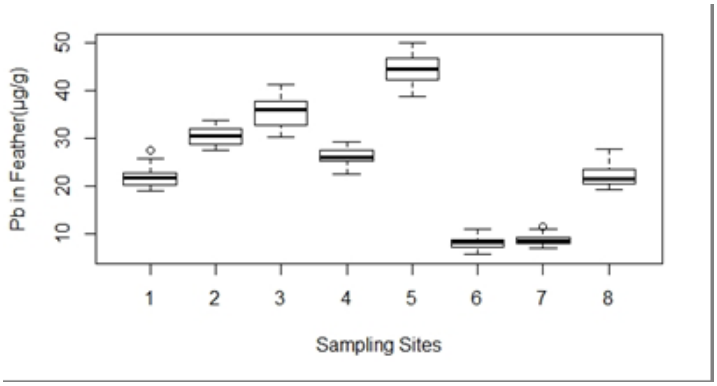

Figure 2. Box-plot of Pb (µg/g) in feather samples of cattle egrets.

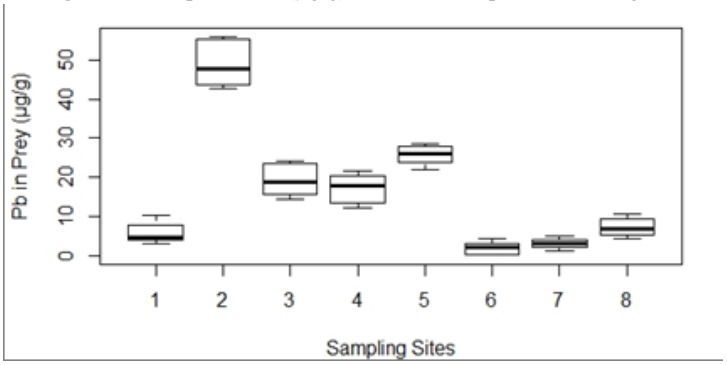

Figure 3. Box-plot of Pb (µg/g) in prey samples of cattle egrets.

3.4. Habitat differences of metal contamination and source identification.

The mean concentration of Pb in soil is relatively similar at different sites of present study. But in some places like Talcherand Hirakud, the concentration is higher due to the geochemistry of parent Rock material. The sampling site of Chilika is the North part of the lagoon where River Daya and its canals end and covered by the runoffs of the river. So this may be the reason for high Pb concentration in the sediments [14–16]. The heronry of site 2 is around 300 mtrs away from the Hirakud Reservoir present in Western Odisha. Those regions are well known for their mineral depositions. Jharsuguda, Sambalpur and Bargarh districts have thermal power plants as well as coal refineries. This may be the cause of metal deposition at the sediment. Thisis affecting the soil organisms also and we found a high concentration of Pb from the prey samples as well as in feather samples. The sampling site of Bhadrak is few kilometers away from the city. This is a pure large agricultural area surrounding the heronry. We found metal in similar patterns in soil pray and feathers. The use of various pesticides and fertilizers may be the cause of metal deposition in soil, prey and feather samples of birds [17–22]. The sampling site of Chandaneswar is a coastal region of North East Odisha. We found comparative less amount of Pb in soil, pray and feather samples in this region with a similar pattern. This place is a rural area and not polluted from external sources but it has a high anthropogenic effect due to the tourist place Digha nearer to this area. Talcher is an urban area with a high coal mining effect. Due to the high pollution effect from coal mining and industries, the accumulation of Pb is very high in cattle egrets here [23]. The highest amount of lead with 45µg/g found here from feathers of cattle egret. The Daringbadi sampling site is a rural and nearly undisturbed area. Pb deposition in soil is comparatively less. The Pb concentration in pray sample is lowest here and accumulation in birds is also lesser than other sites. Titlagarh sampling site is an urban area with a high temperature effect. Less amount of Pb concentration was found in soil. But for prey and feather sample the accumulation of Pb was found to very low. Only urban pollution was obserbed there. There are no industrial pollution effects for causing high accumulation of lead [24]. Koraput is a place of high mineral deposit but Pb is not so dominating in that soil. But in the prey sample, Pb is less but in birds the accumulation is high. Geophagy may be the reason for a higher concentration of metal found in feathers.

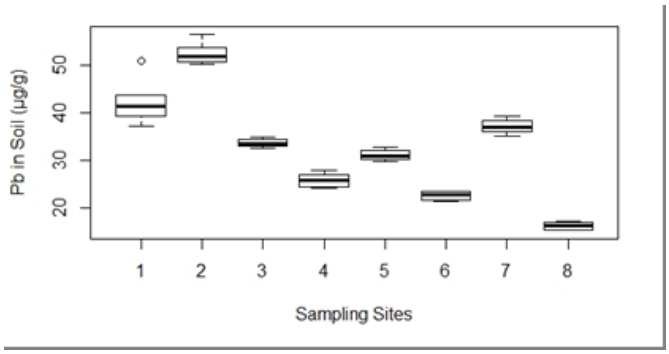

Figure 4. Box-plot of Pb (µg/g) in soilr samples of cattle egrets habitats.

3.5. Comparing with literature.

The concentration of Pb in feathers of cattle egret in this study is much higher compared to other studies in USA, Egypt, Turkey and Hungary [10,20,21,25,26], India [27] and Pakistan [6].

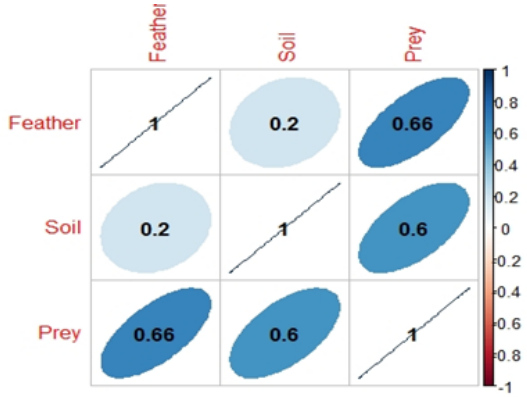

Figure 5. Correlation of Pb among feather, soil and prey samples with colour gradient.

In the present study, there is no sampling point found less than 4µg/g which is harmful to birds [6]. Daringbadi and Titlagarh have more (8-9µg/g) concentration of Pb which is nearly equal with old studies from USA and Egypt [28]. Hirakud,

Chandaneswar, Chilika and Koraput sites are nearly equal with some region of Pakistan [29]. Bhadrak and Talcher sites have the highest accumulation with 35.45µg/g and 44.32µg/g of Pb respectively in feathers which werethe highest among all sites of present study. It is only less than the polluted river site of Pakistan [3] where they found up to 76µg/g of Pb from the feathers of cattle egret.

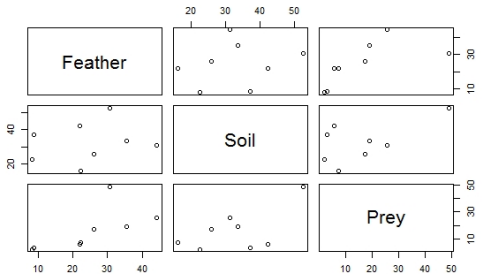

Figure 6. Correlation plot of Pb in feather, soil and prey samples.

Present study describes the positive correlation among soil, prey and feather samples of cattle egret after testing Pearson's correlation with 95% confidence level ($p<0.05$). But correlation plot (Figure 5, 6) describes that soil and prey samples ($t=1.836$, p-value = 0.11) have a high positive correlation between themselves and prey samples have high positive correlation with feather samples ($t=2.177$, p-value = 0.07) of cattle egret. Soil samples also have positive correlation with feather samples ($t=0.499$, p-value = 0.63) of cattle egret. Comparison among means Pb concentration along sampling sites have showed significant differences in concentration of soil ($F=1.196$, $P=0.28$), prey ($F=3.034$, $P=0.07$) and feather ($F=0.942$, $P=0.40$) respectively (Table 1).

Prey samples from Greece [30] and Pakistan [6,11,29] have shown a lower level of Pb than present study. However, Chilika, Titlagarh, Koraput and Daringbadi show a lower concentration of lead in the prey sample than Pakistan [29]. The soil samples from China [31] have only higher concentration of Pb than present study but in Pakistan [6,29], USA [14] and Korea [32] the Pb concentration found to be lower.

Cattle egret feed on roadside insects where Pb deposition is high [33], which may be the cause for a high concentration of Pb found in their body. Insectivorous birds are also affected by feeding habit due to the distribution of invertebrates according to their suitable micro habitats [34,35]. Scheifler et al. [36] described that Pb present in prey samples comes from soil which later used by the birds for their feed in local environment which is also an intense route of Pb exposure. 87 % of contaminants of Pb come from outside sources and 13 % from internal and direct uptake. Pb is a non-degradable metal and its concentration above 4µg/g in feather shows the limit for birds [36]. This will affect the parental sibling's recognition, impaired formulation, locomotion, feeding behaviour and lower chick survival [37]. Pb toxicity affects the animal's nervous system and may result in a decrease in survival growth rate, learning and metabolism [38]. Spahn and Sherry [39] reported that the mortality rate is found higher among Pb exposed nestlings.

Table 1. Descriptive statistics (Mean ± SD and Min - Max) of Pb (µg/g) in soil, prey and feathers of cattle egrets in eight sampling stations of Odisha.

	Soil		Prey		Feather	
	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max
Chilika	42.33±4.83	37.1-51.03	5.29±2.77	3.21-10.49	21.91±2.22	18.9-27.5
Hirakud	52.46±2.83	50.26-56.26	48.83±5.68	42.67-55.76	30.55±2.02	27.51-33.73
Bhadrak	33.61±0.84	32.47-34.8	19.22±4.33	14.4-24.1	35.45±3.12	30.1-41.1
Chandaneswar	25.78±1.45	24.18-27.9	17.31±4.05	12.4-21.78	25.94±1.83	22.58-29.13
Talcher	31.09±1.15	29.8-32.7	25.73±2.43	22.1-28.43	44.32±3.08	38.61-49.86
Daringbadi	22.45±0.89	21.29-23.4	2.04±1.65	0.21-4.43	8.19±1.4	5.6-11.02
Titlagarh	37.06±1.5	35.11-39.25	3.18±1.34	1.23-4.98	8.75±1.31	6.95-11.41
Koraput	16.11±0.78	15.13-17.01	7.38±2.44	4.56-10.85	22.14±2.26	19.21-27.6
Site Differences*	F=1.196		F=3.034		F=0.942	

*Overall differences were tested by one way ANOVA of variance significant at $p<0.05$

4. CONCLUSIONS

Present study described biomonitoring of Pb in feather, prey and soil sample from contaminated wetland habitats of cattle egret. It covers the total Odisha state with eight sampling sites of Eastern India. Association of heavy metal in the environment is due to anthropogenic activities as well as due to various natural hazards. Cattle egret is a widespread species having high Pb accumulation potential from food contamination. Toxicological assessment from feathers is a non-destructive way to describe metal pollution and can be a useful tool in long term environmental health monitoring assessment. This study describes that the metal contamination in the surrounding environment is affecting the cattle egret through food chain. So this habitat contamination can affect other residential birds as well as migratory birds coming to that wetland habitat. High concentration of metal in habitat may be the reason for reproductive failure which leads to population decline among native and migratory birds. Conservation measures should be taken and implemented for controlling the decline in avian population.

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