NON-INVASIVE BIO-MONITORING INVESTIGATION OF TOXIC TRACE ELEMENTS IN REMIGES (FLIGHT), CONTOURS (BODY), AND RECTRICES (TAIL) FEATHERS IN *BUBULCUS IBIS* (CATTLE EGRETS)

M. A. Sheikh[1,2], N. A. Abbasi[1]*, G. Mustafa[2], M. H. Zaman[2,3], M. Majid[2] and S. R. Ahmad[2]

[1]WWF-Pakistan, Ferozpur Road Lahore, 54600, Pakistan
[2]College of Earth and Environmental Sciences, University of the Punjab, Lahore, 54590
[3]Department of Environmental Engineering, Kyungpook National University, Daegu 702-701, Korea
Corresponding author Email: abbasi_akhtar4045@yahoo.com

Highlights:

- Toxic trace elements were examined in the Remiges, Contours, and Rectrices of Cattle Egrets.
- In general, Pb was highest in the feathers of cattle egrets, followed by Cd, Cu, and Cr.
- Hg and As were not detected in all samples.
- Primaries reflect the highest contamination of trace elements.
- Statistical Analysis unveiled non-significant among the study samples.

ABSTRACT: The constant usage of trace elements in the environment can elucidate harmful health effects on sensitive species i.e. Birds residing on higher trophic levels. The current study is to investigate and compare toxic trace elements. accumulation patterns. Cd, Cr, Pb, Cu, As, and Hg in Remiges (Primaries, Secondaries, Tertiaries), Contours (body), and Rectrices (tail) feathers of *Bubulcus ibis* (Cattle Egret) sampled from Lahore, Pakistan. Pb, Cd, Cu, and Cr were detected with variable concentration levels; Hg and As were not detected in the study. The general trend is Pb>Cd>Cu>Cr trend among the Remiges, Contours, and Rectrices of ***Bubulcus ibis***. Primaries Flight feathers showed the highest concentrations of Pb, followed by Cd, the minimum (min-max) concentrations of Pb, Cd, Cu, and Cr at 0.49(0.48-0.55), 0.23(0.07-0.43), 0.13(0.12- 0.14), 0.07(0.03-0.21), were observed in Contour, Tertiaries, Tertiaries, and Primaries feathers respectively. The values of elements were found non-significant (all $P>0.05$) in different feather types of Cattle Egrets, reflecting their similar distribution. Principle Component Analysis (PCA) based on co-relation matrices also depicted the homogenous distribution of toxic trace elements among different feather types. Compared to previous studies from or near to study site, the current study depicts a relatively lower level of toxic trace metals, which are unanimously distributed among different feather types of cattle egret. However, their environmental presence has significant health consequences for Pakistan's already diminishing avifauna.

Keywords: Remiges. Flight Feathers. Rectrices. Biomonitoring. Non-Invasive. Urban. Heavy Metals.

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INTRODUCTION

With the improvement in technology and the rampant increase in population, developing countries have witnessed increased environmental deprivation and threat to living organisms (Abdullah *et al*., 2015). The Chemical processes or manufacturing in different industries, such as dyeing, food processing, and textile sector, introduce toxic trace elements into the environment (Waseem *et al*., 2014). Moreover, these toxic elements present in the environment have high persistence and long durability (Ali & Khan, 2019). Toxic trace elements in excess are also detrimental as they get stored in tissues and organs of living things. The ability of these toxic metals to not manifest their harmful effect immediately in the exposed organism is the reason for more studies on contamination by toxic trace elements (Jakimska, Konieczka, Skóra, & Namieśnik, 2011). When these metals enter any ecosystem, they eventually become bioaccumulated in organisms, resulting in an increased concentration observed at higher trophic levels (Ali & Khan, 2019). In birds, feathers provide a unique opportunity for Eco toxicologists to indicate bioaccumulation of different environmental pollutants during its growth period (Jaspers, Covaci, Herzke, Eulaers, & Eens, 2019).

Distinctive to birds, feathers serve different purposes, including flight, insulation, waterproofing, camouflaging, and reproduction (Jenni & Winkler, 2020). Birds are known bio-indicators as they are more sensitive to environmental contamination. Moreover, they are long-living species on higher trophic levels (Zaman *et al*., 2022). When birds are exposed to heavy metal contamination, their nervous, reproductive, and endocrine systems are adversely affected, resulting in observed behavioral and physiological changes (Sanderfoot & Holloway, 2017). Metals can get accumulated in the feathers through the following routes: (1) Metals can become part of the diet and will evidently be present in feathers during growth; (2) Metals already present in uropygial glands during preening can also contaminate the feathers (3) Metal contamination through direct interaction within the environment (Abdullah *et al*., 2017). Typically, birds can eliminate these hazardous metals and metalloids from their frames by excreting or depositing them in their glands (Abbasi, Jaspers, Chaudhry, Ali, & Malik, 2015). Birds forage a more significant landmass for food and reproduction, so their exposure is maximum. In literature, water birds were frequently used to assess metals and metalloid contamination present in the environment as they are at the top of the food pyramid (Andrew *et al*., 2019).

Furthermore, it can depict a high level of information around the sampling site, and most importantly, it can also establish if the process of bio-magnification is taking place in the species (Hurford, Schneider, & Cowx, 2010). Age and gender play a role in how these substances accumulate in the organs and tissues of birds. Sometimes these parameters can be ignored, and the sample can be considered homogenous, but there is room for error in this practice. It is also established that determining the exact age or sex of the bird is difficult. Moreover, gathering a large sample size is resource-intensive (Castro, Aboal, Fernández, & Carballeira, 2011). Previously invasive methods were being used, such as using internal tissues to measure the degree of exposure to metals and metalloid contamination (Zaman *et al*., 2022). However, the need for different alternative methods has risen due to ethical, conservational, moral, and practical reasons (Kwok *et al*., 2014). In the recent trend, non-invasive methods such as feathers or excrement are used to assess the extent of environmental contamination (Berglund, 2018). Avian feathers are also used as bio-indicator as they are the preferred method for measuring environmental contamination (Jaspers *et al*., 2019). One of the most helpful aspects is that feathers can be sampled from living and deceased birds without harming them.

Additionally, feathers are easy to collect, store, and transport over large distances. Furthermore, they can be sampled during molting seasons. Feathers are also used to reflect the concentration of toxic trace elements and metalloids in blood during feather growth, either from diet or the deployment of stored metals from its internal organs (Horst, 2022). Feathers are also attached to the birds' bloodstream until they are atrophied from the blood vessels, providing information for long-term exposure to environmental contamination (Lopez-Antia, Ortiz-Santaliestra, Mougeot, Camarero, & Mateo, 2018). Some demerits of using feathers for biomonitoring include feathers need for certified standards and no ideal feather to measure the metal contamination. It is further observed that it is challenging to differentiate between external and internal deposition in feathers. The concentration of these toxic metal and metalloids in feathers are difficult to interpret as their molting strategy and preening activity is varied due to their age (Manjula, Mohanraj, & Devi, 2015). Feathers are washed vigorously with deionized water and acetone to remove exogenous metal contamination (Borghesi *et al*., 2016). It has been established that pollutants can only reach feathers through the bloodstream during their growth, highlighting the importance of understanding feather growth. The growth of feathers is species-dependent and can vary in different parts of an individual. However, the growth rate for the length of feathers is nearly uniform (Pacyna-Kuchta, Jakubas, Frankowski, Polkowska, & Wojczulanis-Jakubas, 2020). Feathers can provide a depiction of exposure over an extended period. Additionally, it is important to consider that the molting season of birds is non-synchronous, resulting in variations and differences in the timing of their molts.

Furthermore, the contaminant load will be higher in slow-growing feathers than in fast-growing feathers (Bortolotti, 2010). It is known that the accumulation of metals depends upon the contaminant's chemistry and the location of the feather being discussed. Primaries are preferred more than other feathers as they represent the molting fully (Markowski *et al*., 2013). Rectrices and Contours have also been used frequently to assess bird exposure to pollutants. However, the literature showed that Secondaries and Tertiaries are less preferred because of their molting pattern. The present research aims to evaluate the concentration of toxic metals and metalloids in different feather types, Viz. Primaries, Secondaries, Tertiaries, Contour, and Rectrices of a semi-aquatic bird species, the *Bubulcus ibis* (cattle egret).

METHODS AND MATERIALS

Sample Collection: A total of five individual cattle egrets were collected from Manga Mandi. It is already established that cattle egret is a cosmopolitan species with a habitat in agricultural fields with nesting communities mostly around swamps and riversides. It is mainly considered an invertebrate predator as it mostly feeds on insects, ants, moths, etc. (Abdullah *et al*., 2017). The feathers were selected to analyze their heterogeneous exposure to the toxic trace elements concentrations in the external environment. Feather samples were taken only from the adult birds from sites to minimize the bias in metals accumulation due to age differences. A non-invasive sampling technique was employed to extract samples from the species. The sampled birds were promptly released back into their natural environment. Birds were captured using mist nets, and approximately

1-3g of desired feathers (including contours, rectrices, and remiges (primaries, secondaries, tertiaries) were collected from a sample of five Bubulcus ibis (cattle egret) birds at the sampling site. Each type of feather was stored in separate plastic zip-locked bags. The collected samples were then transported to the Environmental Biology and Ecotoxicology laboratory at the University of the Punjab, Lahore. Feather samples were stored in a refrigerator at 4 °C to prevent any potential contamination from the external environment prior to Analysis. Moreover, trained individuals handled all the feather samples, and the sampling process strictly adhered to international standards for using living organisms in research.

Analysis of Toxic Trace Elements: To assess the concentration of heavy metals, an innovative non-invasive approach involving the utilization of feathers was employed. Due to the developmental characteristics of juveniles, only adult feathers were collected for analysis (De la Puente & Elorriaga, 2012). Research indicates that diverse sections of feathers may exhibit distinct levels of heavy metal contamination (Markowski *et al*., 2013).

To eliminate extraneous contamination, a meticulous procedure was followed. Feathers underwent a thorough cleansing process involving sequential washes with tap water, ultrapure water, and acetone. Subsequently, the samples were air-dried for 24 hours at 80 °C and were safely stored in polyethylene bags within a refrigerator until analysis. All chemicals utilized in the analysis were of analytical grade or higher quality, primarily sourced from Merck, Germany. Throughout the study, solutions were meticulously prepared using bidistilled water from a Sartorius arium 611VF system.

For calibration purposes, ICP Plasma CAL-calibration solution, supplied by SCP-SCIENCE, with a concentration of 1000 mg L^{-1} was employed. Calibration curves were meticulously constructed for each individual metal, encompassing a range of concentrations including 0.1, 0.2, 0.5, 1, 2, 5, and 10 ppm, utilizing standard solutions. Rigorous quality control measures were implemented by employing a standard reference material (Human hair, CRM, ERM-DB001) obtained from the Institute for Reference Materials and Measurements, ensuring the accuracy of the sample results (Abdullah *et al*., 2015). The recovery levels of the standard samples during the digestion process exhibited a variance between 89% and 102%.

The digestion procedure was facilitated using Teflon tubes within a CEM Mars Express microwave system. Each tube contained 0.25 g of the feather sample, 4 mL of 65% HNO3, and 1 mL of 30% H2O2 solution. Digestion was conducted within a 1600-W microwave system at a temperature of 200 °C for a duration of 30 minutes. Upon completion, the solutions were adjusted to a final volume of 50 mL using ultrapure water.

Subsequent elemental analysis encompassed the metals As, Cd, Cr, Cu, Hg, and Pb. This analysis was performed utilizing an ICP-OES (inductively coupled plasma optical emission spectrometer, Varian 720 ES), and the concentrations were quantified in parts per million (μg/g; ppm) based on dry weight. The recorded results for elemental analyses represented the mean of triplicate measurements. The limit of detection values (LODs) were established at 95 μg/L for As, 2 μg/L for Cd, 65 μg/L for Cr, 16 μg/L for Cu, 29.5 μg/L for Hg, and 16 μg/L for Pb..

Statistical Analysis: Principal Component Analysis (PCA) was carried out using MVSP (trial version 3.22) instead of IBM SPSS (Version 26), which was used for the statistical analysis. Levene's test was utilized to determine the homogeneity of variance, and the Shapiro-Wilk test was used to determine normality. Furthermore, Q-Q plots were used to corroborate the data's normality, adding evidence to the theory of normal distribution. Initial calculations for all variables were done using descriptive statistics. To compare how hazardous trace element accumulation varied between different tissues, a parametric one-way ANOVA was employed. The levels of harmful trace elements in various feathers were visually represented and made easier to compare using bar graphs. The distribution of axes was also determined using PCA, which was also used to investigate the relationship between harmful trace elements and feathers. The orientation of the feathers and harmful trace elements on the first two axes of the PCA was shown by the ensuing PCA biplots.

Co-Relation Matrix: The Co Relation matrix in Table 2 represents pairwise similarities or correlations among distinct feather attributes, namely Primaries, Secondaries, Tertiaries, Rectrices, and Contour. The attribute Primaries demonstrates a strong positive correlation (approx. 0.999) with Secondaries, indicating a high degree of similarity between these two feather features. Tertiaries exhibit substantial positive correlations with Primaries (approx. 0.976) and Secondaries (approx. 0.967), indicating significant associations between Tertiaries and these proximate feather attributes. Rectrices manifest pronounced positive correlations with Primaries (approx. 0.998), Secondaries (approx. 0.994), and Tertiaries (approx. 0.989), underscoring robust interrelationships among these feather characteristics. Contour displays appreciable positive correlations with Primaries (approx. 0.973), Secondaries (approx. 0.981), and Rectrices (approx. 0.954), suggesting potential connections between Contour and these respective attributes.

RESULTS AND DISCUSSION

The current study investigated heavy metal contamination in various cattle egret feathers. Distinct concentration patterns were observed across feather types, indicating exposure and physiological process variations. This comprehensive Analysis contributes to biomonitoring efforts and underscores the need for ongoing monitoring and conservation practices to safeguard avian health and ecosystems in urban areas. Overall, the current findings emphasize the importance of targeted conservation strategies based on specific feather types to mitigate risks associated with heavy metal contamination.

Exogenous contamination of feathers with Cd, Pb, Cr, Hg, and As occurs through direct contact with contaminated surfaces or ingesting prey items that have accumulated these elements (Sun *et al*., 2020). Studies have shown that feathers, especially remiges, and rectrices, can reflect the levels of these elements in the surrounding environment (Longrich, Tischlinger, & Foth, 2020). Monitoring the concentration and distribution of these elements in feathers, considering their interaction with preen oil, allows for identifying contamination sources and assessing potential risks to bird populations (Martínez, Crespo, Fernández, Aboal, & Carballeira, 2012).

Aquatic bird feathers previously exhibited minimal mercury concentrations, as documented in prior studies (Malik & Zeb, 2009). Conversely, falcon feathers demonstrated comparatively higher mercury levels, reaching 3.09 ppm across six distinct study sites (Lodenius & Solonen, 2013b). The study investigation reveals that sampled environment exhibits lower mercury exposure, potentially due to geographical or species-specific variations. The Minamata Convention, ratified by Pakistan in 2020, has resulted in restricted mercury production and stringent environmental regulations, significantly reducing mercury and arsenic usage. The findings underscore a substantial decline in releasing these toxic elements into the local ecosystem.

Mercury accumulation in bird feathers occurs during molting, as it is excreted from the bloodstream and immobilized within the keratin structures of feathers (Mishra *et al*., 2019). Once feathers form, the blood supply to them diminishes, trapping (Movalli *et al*., 2017). Subsequently, dietary intake influences mercury levels in feathers. Based on this premise, we hypothesize that earlier-formed feathers may contain higher mercury concentrations compared to subsequent feathers.

The previous study conducted in Lahore, Pakistan, reported notably higher mean concentrations of arsenic (in ug/g) in cattle egret samples i.e., 21.4 ± 4.2 μg/g (Abdullah *et al*., 2015). In contrast, the current study revealed no detectable levels of arsenic. This divergence can be attributed to temporal variations, as the previous study was conducted from 2015 through 2016, characterized by extensive utilization of arsenic in the industrial and agricultural sectors.

Groundwater contamination serves as a major pathway for arsenic uptake in cattle egrets, significantly contributing to their exposure (Ullah, Ahmad, Majeed, & Sohail, 2021). Notably, the prohibition of arsenic used in fertilizers and agricultural products has been enforced in Pakistan due to its well-established detrimental effects (Islam *et al*., 2017). Ongoing research efforts are focused on assessing groundwater arsenic levels and implementing preventive measures to mitigate its presence.

The combined efforts corroborate the study's finding that the concentration of arsenic is below the detection limit (BDL), as they represent a significant decrease in the amount of arsenic that organisms are exposed to. In addition, it's important to consider a variety of biological factors, such as gender, age, and ecological factors, such as proximity to pollution sources, reproductive status, and bird habitat, which may contribute to the observed variations in arsenic levels among cattle egret populations (Lodenius & Solonen, 2013a).

Variation in Heavy Metal Contamination among Feather Types: Overall, the present findings emphasize the need of considering various feather types when evaluating heavy metal contamination in birds. Feathers may act as possible exposure indicators and offer important information about the sources and pathways of pollution due to the diversity in heavy metal concentrations among different species of feathers. These results provide a foundation for more research on the effects of heavy metal contamination on the health and preservation of cattle egret populations and help us comprehend the ecological dangers connected to heavy metal pollution. The variety could be linked to a number of things, such as variations in feather growth patterns, feather age, exposure to contaminating environmental sources, and the feather's function as a tissue and organ protector.

Primary Feathers: Cadmium (Cd) had a concentration of 0.25 ppm, which was somewhat higher than copper's (0.21 ppm) concentration. The concentration of chromium (Cr) was lower, at 0.07 ppm. Surprisingly, Lead (Pb) showed the highest concentration out of all the metals examined, at 0.57 ppm. Lead (Pb) was the heavy metal with the highest concentration in primary feathers, which had moderate levels of contamination. This suggests a potential risk of lead toxicity in cattle egrets. The species' foraging behavior, which involves consuming contaminated prey or exposure to polluted habitats, may contribute to the higher exposure to lead (Shivanna, 2020). Primary feathers location and proximity to the external environment during flight and foraging activities likely contribute to lead accumulation (Thongcharoen, Robson, & Keithmaleesatti, 2018). Lead poisoning can lead to profound health issues, manifesting as conditions like depression, paralysis, leg or wing weakness, and neurological harm, including damage to the brain (encephalopathy) (Varagiya, Jethva, & Pandya, 2022). The presence of lead in feathers can have both endogenous and exogenous origins. Endogenously incorporated lead reflects its content in the blood as it is distributed to various internal organs and tissues, including muscles, through the bloodstream (Xia, Ma, Yi, & Lin, 2021). However, its concentration decreases over time, with significant accumulation occurring in bone tissue and feathers during their initial development (Pacyna-Kuchta, 2023). Feathers irrigated by blood vessels during growth allow for the mobilization and deposition of lead, which can associate with keratin and become immobilized (Zebral, Costa, de Souza, & Bianchini, 2022). This indicates contamination by lead during these feathers' relatively short growth period. Exogenous lead, on the other hand, may serve as an indicator of air pollution. Industrial, mining, and transportation activities contribute to environmental lead deposition. Lead from the air becomes a significant receptor and can deposit on feathers in sedentary species, indicating air pollution (Su, 2014). Therefore, the presence of lead in primary feathers can reveal information about recent exposure and levels of atmospheric pollution.

The differences in metal content across the various types of feathers are highlighted by these observations. The findings shed important light on how these metals could accumulate and spread among avian species, emphasizing the significance of taking feather type into account when researching metal contamination.

Secondary Feathers: Cadmium (Cd) had a concentration of 0.30 ppm, which was slightly greater than the concentration of copper (Cu), which was 0.15 ppm. Lead (Pb) and chromium (Cr) both showed concentrations of 0.53 ppm and 0.15 ppm, respectively. Elevated cadmium (Cd) concentrations in secondary feathers suggested possible exposure hazards (Gao *et al*., 2021).

Secondary feathers showed similar patterns of heavy metal contamination as primary feathers. Cadmium accumulation may be influenced by migration patterns, foraging habits, and proximity to hazardous sites. Birds' physiological functions are impacted by cadmium's high toxicity (Fu, Wang, Wang, Yu, & Zhang, 2014). Concentrations in feathers were higher than threshold values deemed potentially hazardous to bird populations (Dauwe, Janssens, Pinxten, & Eens, 2005). Elevated cadmium levels are a result of anthropogenic and industrial sources.

Continued monitoring and mitigation efforts are crucial to protect the health of cattle egrets and other avian species in these areas (Thakur & Kaur).

Secondary feathers displayed similar patterns of heavy metal contamination as observed in primary feathers (Hurford *et al*., 2010). Cadmium (Cd) exhibited elevated concentrations, highlighting a potential exposure risk. The accumulation of cadmium in secondary feathers could be influenced by factors such as migration patterns, foraging behavior, and the proximity to contaminated water bodies or agricultural areas where pesticides and fertilizers containing cadmium might be utilized (Devalloir *et al*., 2023). The higher accumulation of cadmium and lead in secondary feathers might be attributed to their proximity to primary feathers during flight, where heavy metals can transfer from the adjacent primary feathers.

Tertiary Feathers: Copper (Cu) had a concentration of 0.13 ppm, and Cadmium (Cd) exhibited a concentration of 0.23 ppm. Chromium (Cr) showed a concentration of 0.17 ppm, while Lead (Pb) had a concentration of 0.56 ppm.

Tertiary feathers displayed comparable contamination levels to primary and secondary feathers, with elevated copper (Cu) concentrations. Despite its essential micronutrient status, excessive copper exposure can elicit toxic effects, warranting further investigations to ascertain the exceedance of adverse thresholds and discern potential sources of copper contamination in cattle egret habitats. These findings highlight the significant contribution of tertiary feathers to heavy metal accumulation, likely attributed to their anatomical positioning and exposure to environmental contaminants. It is important to note that copper deposition primarily occurs endogenously via preen oil-mediated transport and deposition.

Excessive copper exposure can induce reproductive, respiratory, gastrointestinal, hepatic, endocrine, and ocular damage and heightened cancer susceptibility. Considering the permissible limits in various environmental matrices, the observed concentrations pose severe reproductive and physiological health risks for avian populations. Implementing stringent measures to mitigate copper contamination is pivotal for preserving the well-being of cattle egrets and similar avifauna.

Chromium (Cr), is not considered as an essential element for animals. Further it is reported that it can cause deleterious effects on reproductive health in different avian species, its presence was frequently detected in the samples. Comparing the current dataset with other documented studies worldwide, it becomes evident that severe chromium contamination exists in current study area.

Similarly, chromium concentrations in avian feathers have been reported in previous studies conducted in Pakistan and China. The high concentrations of chromium in the study area can be attributed to its extensive use in the tanning process of the leather industry, leading to the disposal of chromium salts as effluent and sludge in the environment. Biota, including cattle egrets, can uptake these chromium compounds through the food chain, thus explaining their elevated levels in the feathers. The uptake of cadmium (Cd), chromium (Cr), and lead (Pb) in bird feathers may originate from secretions of the uropygial gland and the nasal gland, with the keratin binding affinity of these heavy metals potentially enhancing their accumulation.

High levels of copper and chromium were found in the tertiary feathers of cattle egrets, which highlights the significance of identifying and reducing heavy metal contamination in their environments. The possible health concerns to populations of cattle egrets and other bird species must be reduced using effective procedures.

Tail Feathers (Rectrices): The concentration of copper (Cu) was 0.22 ppm, whereas the concentration of cadmium (Cd) was 0.27 ppm. Lead (Pb) had a value of 0.53 ppm, and chromium (Cr) had a concentration of 0.16 ppm.

The levels of heavy metals in tail feathers matched the patterns in primary and secondary feathers, which were detected. Cattle egrets may be at risk because lead (Pb) indicated significant contamination levels. Tail feathers serve as indications of long-term exposure to heavy metals and offer important information about the levels of contamination overall because they grow continually and are exposed to environmental influences for a long time. Due to continual exposure to the environment and potential contact with polluted surfaces during perching and nesting, high metal buildup in tail feathers may be explained.

Contour Feathers: Cadmium (Cd) had a concentration of 0.32 ppm, whilst copper (Cu) had a concentration of 0.20 ppm. Lead (Pb) had a concentration of 0.49 ppm while chromium (Cr) had a concentration of 0.10 ppm.

The contour feathers, which are identical to the other feather types in terms of pollution, were also noticeable. The study's heavy metals showed that cadmium (Cd) had the highest concentration, which might be harmful if inhaled. Cattle egrets' exposure to the hazardous heavy metal cadmium over an extended period of time might negatively influence their ability to reproduce and the dynamics of their population, raising questions about the health and wellbeing of the species as a whole. This study raises the possibility that the contour feathers, which cover the majority of the bird's body surface, may act as a storage area for heavy metal buildup, potentially indicating prolonged exposure to contaminated settings.

Inter Feathers Difference: Metal buildup and dispersion patterns in avian species are useful insights gained from the examination of metal concentrations in various feather types. Our research found that the rectrices, contour feathers, secondary feathers, tertiary feathers, and primaries all had different metal concentrations. The levels of exposure, the methods of absorption, and the affinity for binding with feather proteins are only a few of the variables that can explain these variations.

Table 1: Illustration of targeted toxic trace elements concentrations in various feathers (mean ± S.D., min-max), i.e., Primaries, Secondaries, Tertiaries, and Contour (μg/g wet weight) of cattle egret (Bubulcus ibis) collected (n=5) from the premises of Lahore.

Feathers	**Cu**	**Cd**	**Cr**	**Pb**
	Mean ± S.D.	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**
	Min-Max	**Min-Max**	**Min-Max**	**Min-Max**
Primaries	0.21 ± 0.03	0.25 ± 0.04	0.07 ± 0.08	0.57 ± 0.10
	(0.21 - 0.25)	(0.24 - 0.30)	(0.03 - 0.21)	(0.56 - 0.70)
Secondaries	0.15 ± 0.02	0.30 ± 0.10	0.15 ± 0.04	0.53 ± 0.09
	(0.15 - 0.18)	(0.25 - 0.45)	(0.13 - 0.22)	(0.50 - 0.61)
Tertiaries	0.13 ± 0.03	0.23 ± 0.16	0.17 ± 0.06	0.56 ± 0.09
	(0.12 - 0.14)	(0.07 - 0.43)	(0.12 - 0.22)	(0.55 - 0.68)
Rectrices	0.22 ± 0.03	0.27 ± 0.09	0.16 ± 0.11	0.53 ± 0.11
	(0.21 - 0.26)	(0.20 - 0.38)	(0.09 - 0.34)	(0.48 - 0.69)
Contour	0.20 ± 0.03	0.32 ± 0.05	0.10 ± 0.08	0.49 ± 0.06
	(0.19 - 0.23)	(0.31 - 0.38)	(0.01 - 0.17)	(0.48 - 0.55)
Test for significance F (p)	**20.347 (0.000)**	0.414(0.795)	0.753(0.578)	1.159 (0.385)

Different exposure levels and the metals' preferences for binding to particular proteins in feathers can account for the observed variability in metal concentrations within the primary feather type. Due to their intermediate quantities, copper (Cu) and cadmium (Cd) may have undergone similar environmental exposure levels or uptake mechanisms. Comparatively, Chromium (Cr) showed a lower concentration, indicating either a decreased exposure to this metal or a limited uptake in the primaries' feathers. The lead (Pb) concentration was found to be at its greatest level, demonstrating both its pervasiveness in the environment and birds' capacity to absorb it from a variety of sources.

Limitations and Future Prospects: This study has several limitations that warrant further investigation. Firstly, the sample size was relatively small, necessitating larger sample sizes to minimize statistical biases. Additionally, considering ethical and scientific concerns, non-invasive biomonitoring methods should be prioritized for assessing toxic trace element levels. Furthermore, the study focused on a single bird species in a specific location, requiring broader sampling across multiple species and habitats to enhance generalizability. Future research should establish precise relationships between toxic trace element bioaccumulation in different feathers, considering intricate biological and ecological factors. In conclusion, it is critical to consider different feather types when evaluating the level of heavy metal contamination in distinct avian species. The aforementioned restrictions can be overcome by using bigger sample numbers, non-invasive methods, and a wider range of species, which will enhance conservation efforts and increase knowledge of the effects of heavy metals on bird populations and ecosystems.

Table 2: Co-Relation Matrix of metals among different feathers.

	Primaries	Secondaries	Tertiaries	Rectrices	Contour
Primaries	1				
Secondaries	0.999227	1			
Tertiaries	0.975902	0.96657	1		
Rectrices	0.997575	0.994069	0.988722	1	
Contour	0.972711	0.981079	0.898641	0.954205	1

Figure 1: Location map of the Sampling site (Manga Mandi) illustrates the sampling points for cattle egret (Bubulcus ibis) collected from the premises of a metropolitan city, Lahore, Pakistan.

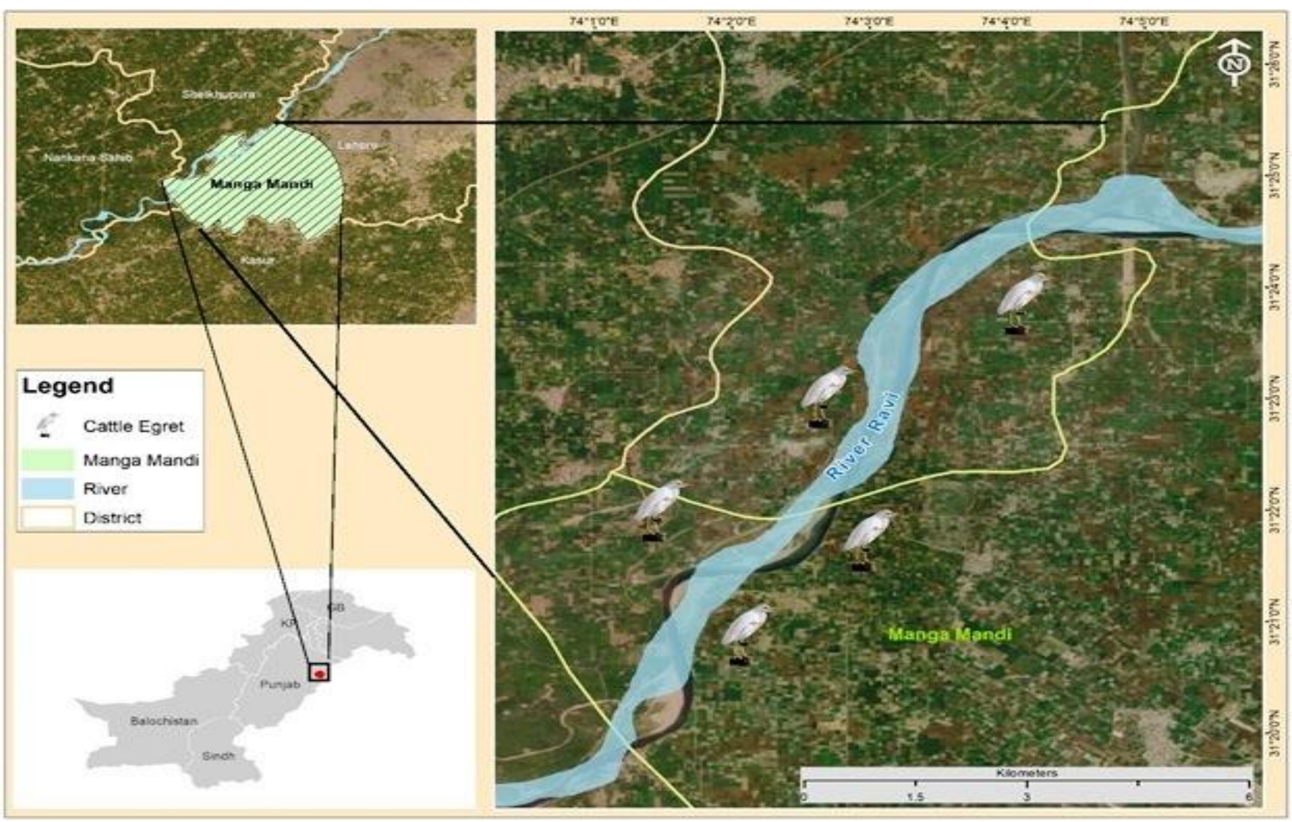


Figure 2: Comparison of concentrations (µg/g) of targeted toxic trace elements in different Feathers of cattle egrets (*Bubulcus ibis)* collected from the premises of a metropolitan city, Lahore, Pakistan.

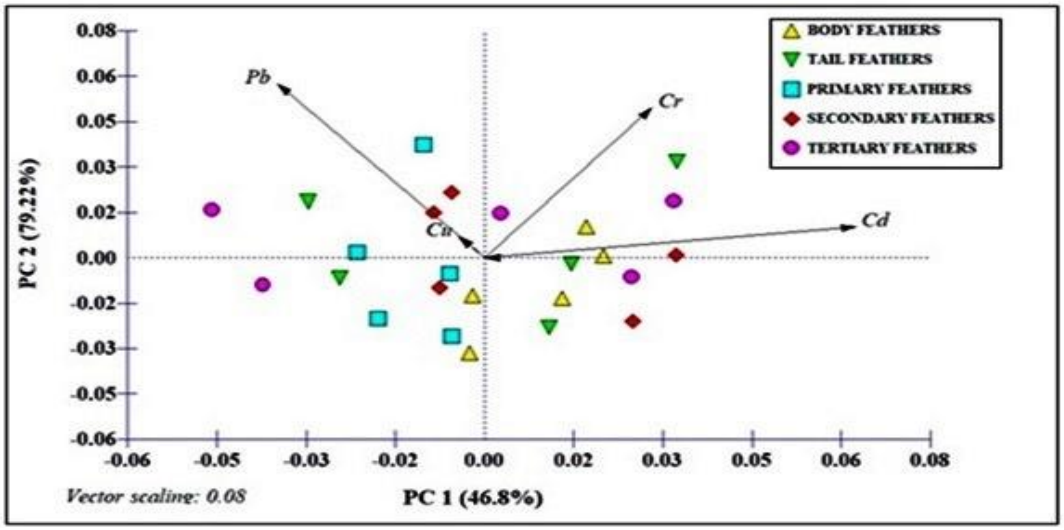
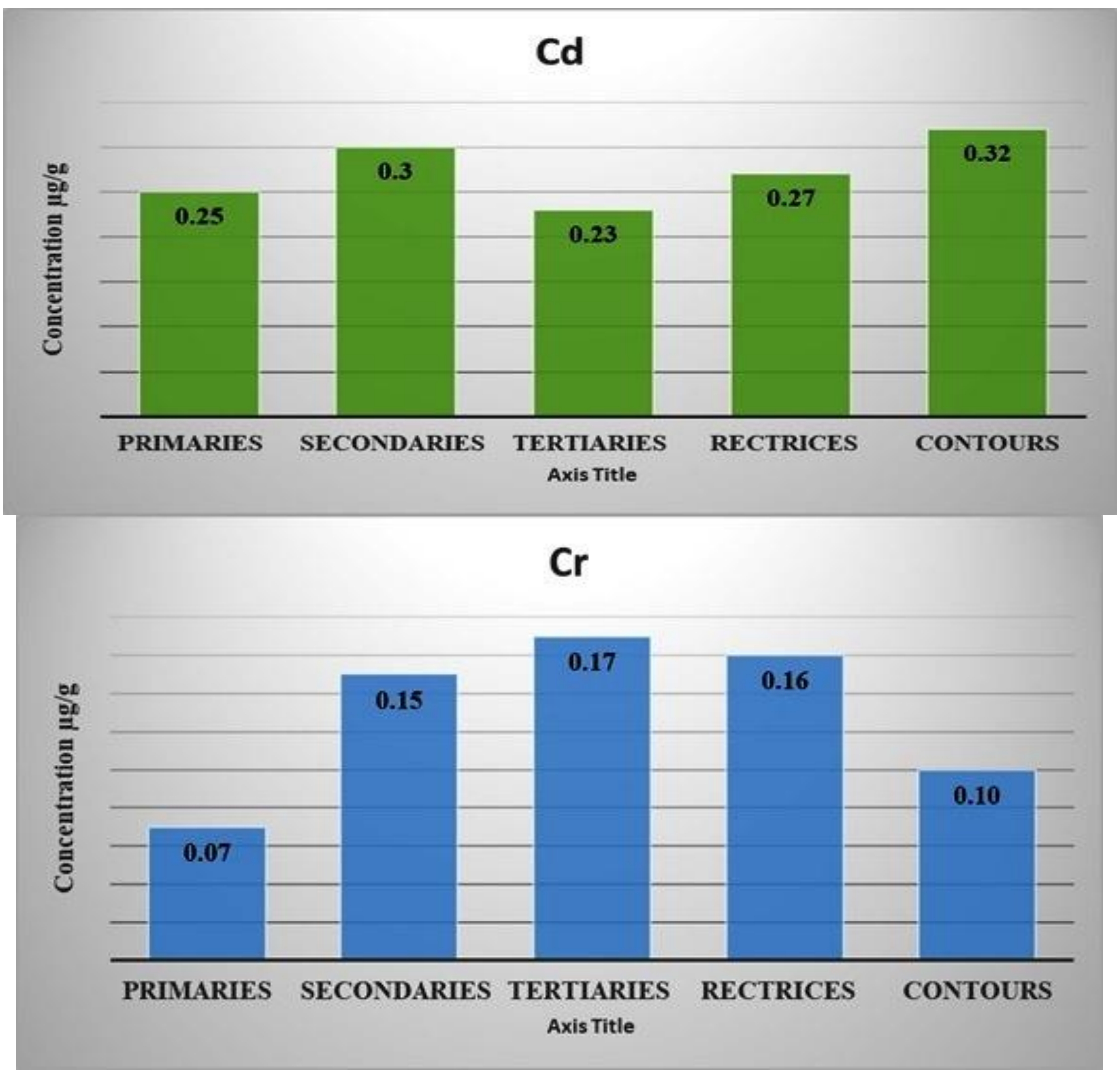
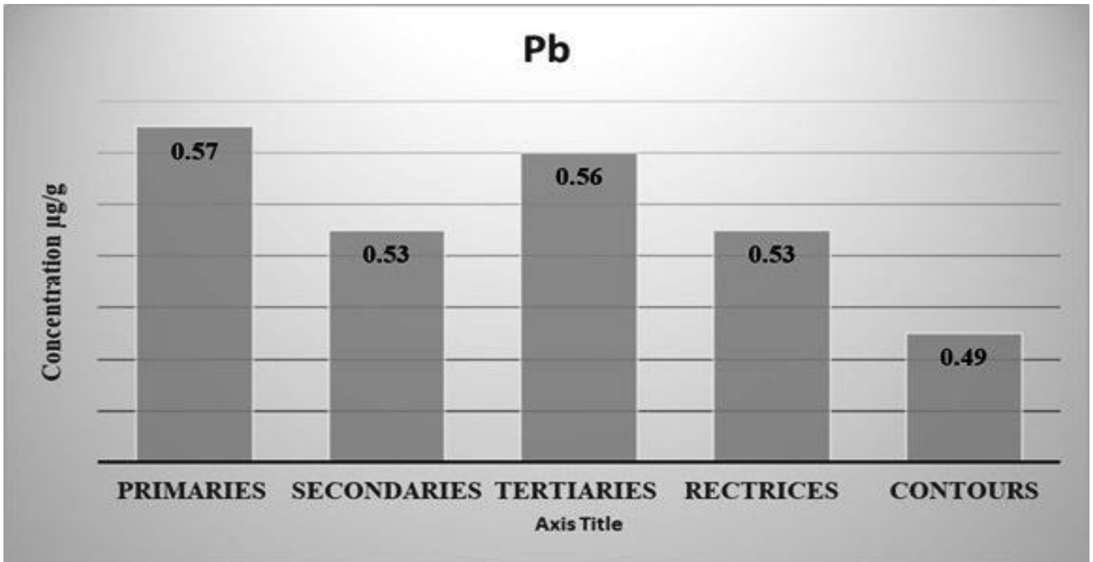


Figure 3: Euclidean biplots of Principal Component Analysis including first two principal components (PC1 & P.C. 2) illustrating the association of toxic trace elements with different feathers of cattle egret (*Bubulcus ibis)* collected from the premises of a metropolitan city, Lahore, Pakistan.




Pb
Concentration µg/g
0.57
0.53
0.56
0.53
0.49
PRIMARIES
SECONDARIES
TERTIARIES
RECTRICES
CONTOURS
Axis Title

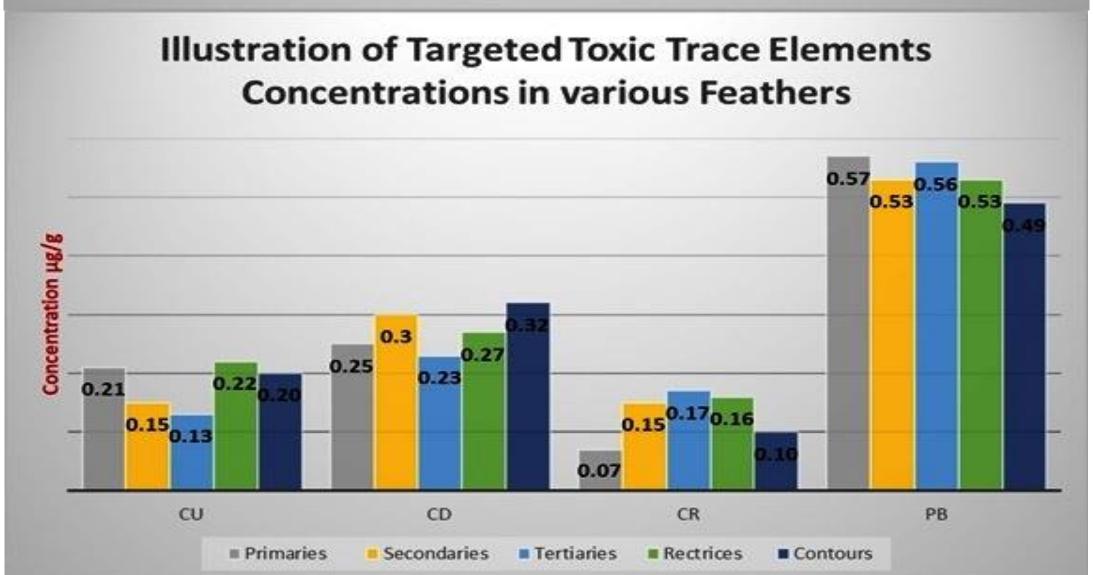
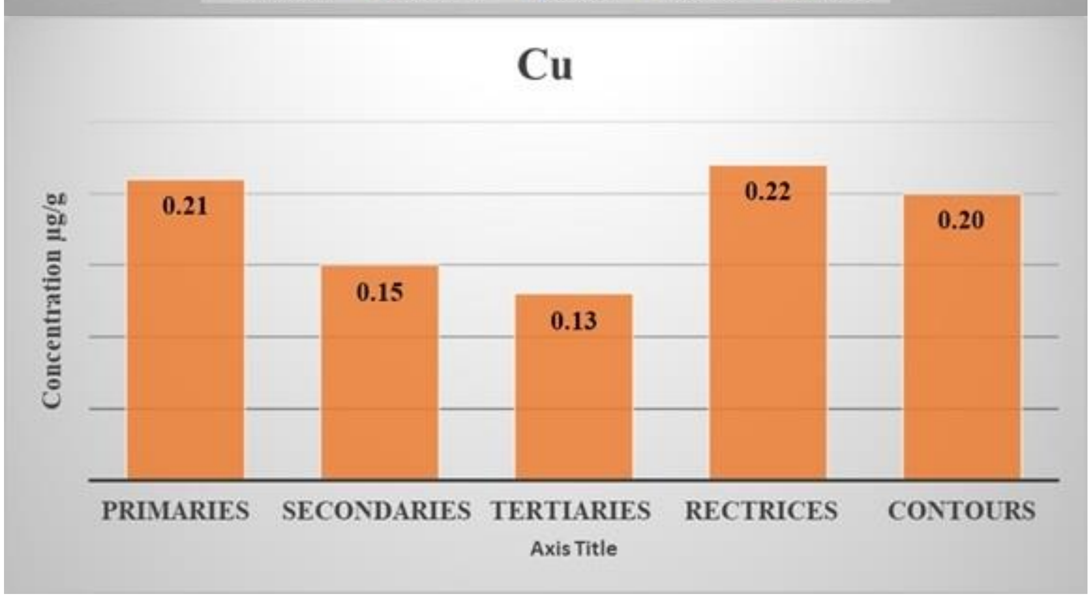


Illustration of Targeted Toxic Trace Elements Concentrations in various Feathers
Concentration µg/g
0.21
0.15
0.13
0.22
0.20
0.25
0.3
0.23
0.27
0.32
0.07
0.15
0.17
0.16
0.10
0.57
0.53
0.56
0.53
0.49
CU
CD
CR
PB
Primaries
Secondaries
Tertiaries
Rectrices
Contours


Cu
Concentration µg/g
0.21
0.15
0.13
0.22
0.20
PRIMARIES
SECONDARIES
TERTIARIES
RECTRICES
CONTOURS
Axis Title

Author's contribution: Muteeb Ahmad Sheikh sampling, literature review, lab analysis, draft preparation. Ghulam Mustafa sampling, lab analysis, draft preparation. Muhammad Hamza Zaman sampling, lab analysis, literature review, draft preparation. Sajid Rashid Ahmad Species Selection and Methodology, Muzaffar Majid draft revision, Map development. Naeem Akhtar Abbasi study design, draft design, and editing, statistical Analysis, project supervision.

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