

## ASSESSMENT OF HEAVY METAL CONTAMINATION IN WATER, SOIL, AND FEATHERS OF *Bubulcus ibis* IN CANAL ECOSYSTEMS OF PAKPATTAN, PUNJAB, PAKISTAN

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### ABSTRACT

Environmental contamination by heavy metals is a leading concern, particularly in aquatic ecosystems where pollutants can accumulate and disrupt ecosystem balance. This study evaluates the presence of manganese (Mn), lead (Pb), copper (Cu), and zinc (Zn) in water, soil, and *Bubulcus ibis* (cattle egret) feathers collected from selected sites in Pakpattan, Punjab, Pakistan. 24 samples comprising (8 soil, 8 water, 8 avian) feathers were obtained from four canal locations. Heavy metal concentrations were analyzed by atomic absorption spectrophotometry, with measured concentrations in samples as follows: Mn (water: 19.56±4.01, soil: 14.01±5.60, feathers: 2.06±0.53), Pb (water: 7.97±1.62, soil: 11.81±1.51, feathers: 10.30±1.57), Cu (water: 7.43±1.04, soil: 6.75±1.16, feathers: 3.09±0.59), and Zn (water: 0.62±0.21, soil: 2.34±0.76, feathers: 7.18±1.32). Results showed significant differences ( $p < 0.05$ ) in concentrations across media for Mn, Cu, and Zn: Zn accumulated predominantly in feathers, while Mn and Cu were highest in water and soil. Pb showed no significant variation ( $p = 0.244$ ). Correlation analysis revealed possible contamination sources and pathways affecting metal distribution. These findings highlight the potential of avian feathers as bio-indicators for monitoring heavy metal pollution and emphasize the necessity for ongoing environmental assessment and conservation efforts.

**Keywords:** Water, Soil, Cattle egret, Heavy metals, feathers

**Keywords:** orange, postharvest, packaging, plastic materials, darkness, storage, shelf life, quality, enzymes

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### INTRODUCTION

Due to industrialization, urbanization, and rapid consumption of fuels by industry, households, and transportation vehicles environmental pollution has increased (Swaileh and Sansur, 2006; Ajibade *et al.*, 2021). Metals are non-degradable and persist in the atmosphere, where they may be bioaccumulated (Kontas, 2007). Heavy metals are highly toxic and lethal to the environment (Duffus, 2002; Jomova *et al.*, 2024).

Lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu), chromium (Cr), zinc (Zn), manganese (Mn), nickel (Ni), etc. are most common heavy metals present in the environment (Tae *et al.*, 2020). Metal toxicity is the reason of oxidative stress in fishes and this stress affects growth and fertility by weakening immune system, causing organ and tissue damage, impairing growth, and impairing fertility (Rahman *et al.*, 2023). These metals disrupt an organism's physiological and metabolic processes, which frequently results in oxidative stress,

decreased fitness, and reproductive problems (Govind and Madhuri, 2014). Anthropogenic activities such as industrial discharge, agrochemicals, electroplating, and fuel emissions are the cause of their environmental presence (Panagos *et al.*, 2018). Pigment production, organic waste, pharmaceuticals, agrochemicals, industrial emissions, plastics, electroplating and fuel emissions all contribute to toxic metal accumulation in environment (Baig *et al.*, 2024).

Numerous biological matrices, including feathers, excrement, eggs, muscles, and internal organs like the liver and kidneys, can be used to measure the accumulation of heavy metals in birds. Both natural and man-made processes, such as urbanization, industrialization, and irrigation techniques, are the sources of these metals, which greatly contaminate Pakistan's environment (Alloway, 1995; Biswas, 2023; Mitran *et al.*, 2024). Aquatic birds experience bioaccumulation as a result of wastewater flow from industrial and residential sources polluting waterways and

agricultural areas. In particular, the use of fertilizers and pesticides containing toxic metals, as well as mining activities, steel mills, tanneries, thermal power plants, and battery production, are the main causes of soil and water pollution in agricultural areas (Amman *et al.*, 2002; Nandakumar *et al.*, 2025).

Among birds, the cattle egret (*Bubulcus ibis*) is a common waterbird that forages in wetlands and agricultural areas, which makes it especially vulnerable to environmental pollutants. It is a useful bioindicator of heavy metal exposure in contaminated environments because of its eating habits, site fidelity, and convenience of feather sample (Malik and Zeb, 2009). While earlier research has looked at the buildup of heavy metals in avifauna around the world, nothing is known about the concentrations and trends of these pollutants in aquatic birds in Punjab, especially in the canal-irrigated agro-ecosystems of Pakpattan. The risk of metal pollution in water, soil, and wildlife is increased by the region's high level of agricultural activity and uncontrolled pesticide usage.

Thus, the current study is to evaluate the levels of four heavy metals in water, soil, and *Bubulcus ibis* feathers taken from specific canal sites in District Pakpattan, Punjab, Pakistan: manganese (Mn), lead (Pb), copper (Cu), and zinc (Zn). The purpose of this study is to assess the metals' spatial distribution and determine whether *Bubulcus ibis* feathers may be used as a non-invasive biomonitoring method for environmental metal contamination.

## MATERIALS AND METHODS

**Study area:** This study assessed heavy metal concentrations in water, soil, and aquatic avifauna in Pakpattan, Punjab, Pakistan, encompassing two tehsils

and spanning approximately 0.674 million acres, is geographically positioned between 30.06° to 30.38° N latitude and 73.03° to 73.36° E longitude. The region experiences a subtropical continental climate, characterized by extreme summer temperatures that can soar to 49°C in May and June. Monsoonal conditions bring annual rainfall ranging from 300 to 400 mm, while winter temperatures in December and January can plummet to freezing levels.

**Sample collection:** A total of 24 samples were collected from four sites in District Pakpattan: Pakpattan Canal, Bakha Canal, Khader Canal, and Dike Chak Moher Singh Canal. At each site, six samples were obtained, including two water samples, two soil samples, and two chest feather samples from *Bubulcus ibis* (cattle egret). This resulted in eight samples for each type across all sites (water: n = 8; soil: n = 8; feathers: n = 8). All samples were collected under an approved animal ethics protocol (Approval No. DR/1003).

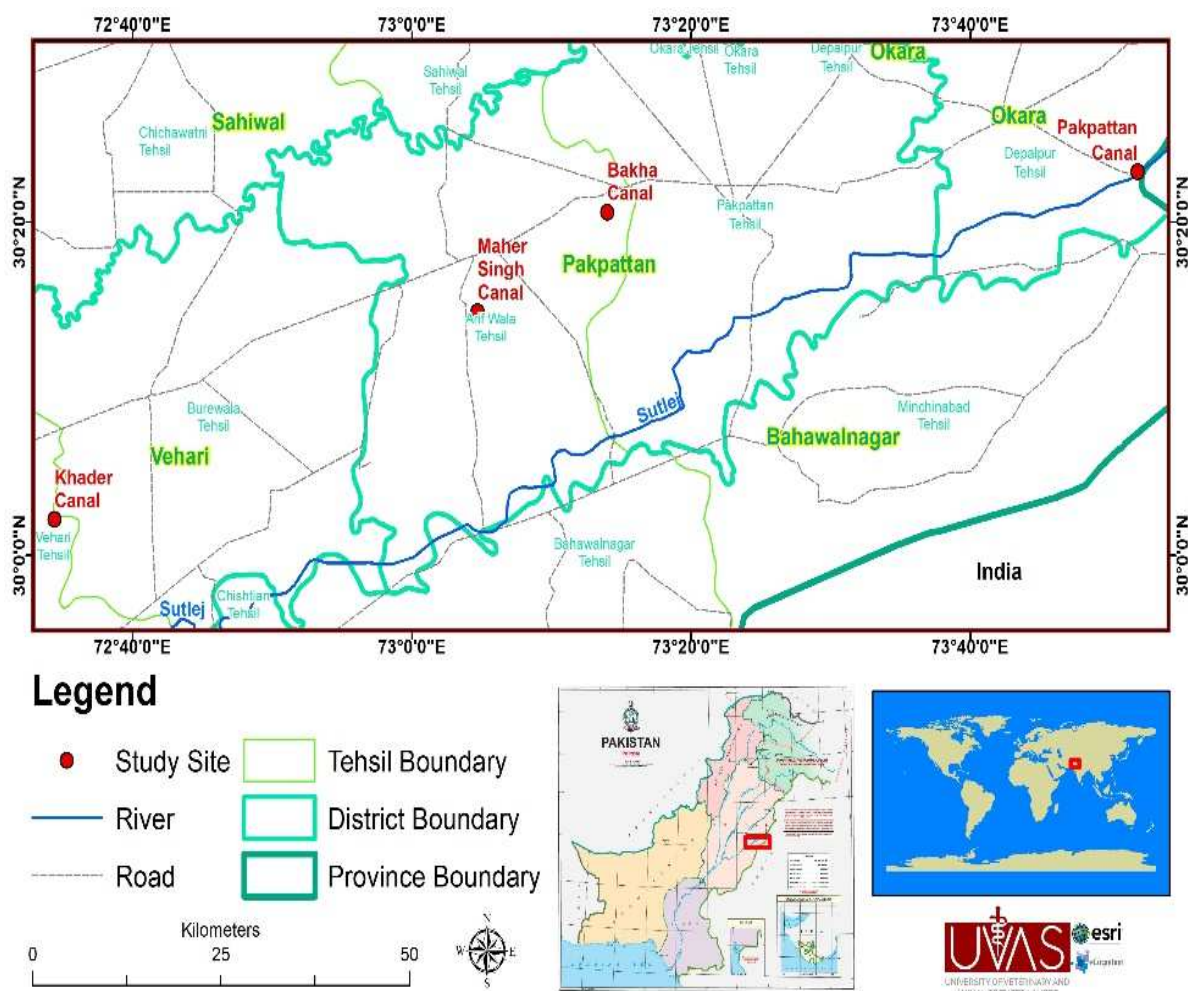
**Table-1: Geospatial distribution of collected Samples**

Sample Locations	Latitude	Longitude
Pakpattan Canal	30°22'60" N	73°52'0" E
Bakha Canal	30°20'34.5"N	73°13'59.3"E
Khader Canal	30°2'8.77" N	72°34'24.28" E
Dike Chak Moher Singh Canal	30°14'37.2"N	73°04'42.5"E

Birds (cattle egrets) were captured by using traps and immediately set free after taking samples. All collected samples were transported to the Department of Wildlife and Ecology Laboratory at Ravi Campus, Pattoki, for further processing and analysis.



**Fig.1. Depiction of Province Punjab, Pakistan on mapLink:** <https://www.nation.com.pk/06-May-2012/south-punjab-larger-in-size-less-in-population>



**Fig.2: Study Area Map of Sampling Sites from District Pakpattan**

**Feathers samples preparation and digestion:** Feather samples were first rinsed with tap water, followed by 1M acetone and triple washing with distilled water to remove contaminants. Samples were air-dried and then dried in an oven at 105°C for approximately 2-3 hours. For easy digestion, feathers were cut into pieces by the use of stainless steel scissors. Feather sample of 0.5 g was transferred to quartz crucibles, and 1.0ml HNO<sub>3</sub> and 0.25ml HClO<sub>4</sub> were added in the crucibles (covered with lid) and digested on hot plate. Initially on low temperature and then gradually increasing the temperature and continued until the mixture became pale yellow. Completely digested samples were filtered and diluted by using 50ml distilled water and stored in sample bottles at -20°C (Nighat *et al.*, 2013).

**Soil samples preparation and digestion:** Samples of soil were allowed to air dry before being pulverized with a mortar and pestle and sieved to a size of 2 mm. After treating a 3 g sample with 3.5 mL HNO<sub>3</sub> and 10 mL HCl, it was placed in a fume hood for the entire night and

heated for two hours at 104°C. For analysis, the solution was filtered, diluted, and kept in plastic bottles.

(Ogundele *et al.*, 2015).

**Water samples preparation and digestion:** 10 ml of water samples were taken and digested with 5ml HCl and 2ml HNO<sub>3</sub>. The samples then were heated at 95°C until a clear solution was obtained. The solution was filtered and cooled. Final volume was adjusted on 50ml (Ashraf *et al.*, 2021).

**Metal Analysis and Quality Control:** Digested samples were analyzed for metal content (Pb, Zn, Cu and Mn) using atomic absorption spectrophotometer (Bankaji *et al.*, 2023). These analyses were performed at Advanced Central Research Laboratory, Department of Chemistry, Lahore College for Women University, Lahore

**Statistical analysis:** IBM SPSS Statistics 20 was used to compute descriptive statistics (means and standard errors). Tukey's HSD test was performed for multiple comparisons. One-way ANOVA was performed to

evaluate significant differences in the concentrations of heavy metals (Mn, Pb, Cu, and Zn) across the three sample matrices (water, soil, and feathers). To investigate the correlations between metals across sample categories, Pearson correlation analysis was used (Chandrasekaran *et al.*, 2015).

## RESULTS

### Comparison with international safety standards:

Heavy metal concentrations were compared against international safety standards. Table 2 summarizes the mean concentrations ( $\pm$ SE) of heavy metals across the three environmental media. One-way ANOVA revealed statistically significant differences ( $p < 0.05$ ) among matrices for Mn, Cu, and Zn. Zn concentrations were significantly higher in feathers, whereas Mn and Cu were

more concentrated in water and soil. However, Pb levels did not show significant variation across sample types ( $p = 0.244$ ).

The optimal ranges for heavy metals in water, soil, and feathers were derived from WHO guidelines (2011, 2017) for water, de Vries (2012) for soil, and Burger (1993) and Dauwe *et al.* (2003) for avian feathers.

**Water:** Pb (7.97 ppm) and Cu (7.43 ppm) were 59% and 272% higher than safety limits, respectively, reflecting industrial/agricultural contamination.

**Soil:** No metal was present outside safe levels, but Pb (11.81 ppm) should be monitored for steady build-up.

**Feathers:** Pb (10.30 ppm) was above bird toxicity levels (6 ppm), reflecting bioaccumulation hazards.

**Table 2: Heavy metal concentrations (ppm) with optimal range comparison.**

Metal	Matrix	Mean $\pm$ SE	Optimal Range*	% Above Optimal	Significance
Pb	Water	7.97 $\pm$ 1.62	1.0-5.0 $\mu$ g/L	59% $\uparrow$	Exceeded
	Soil	11.81 $\pm$ 1.51	50-300 mg/kg	Below	Safe
	Feathers	10.30 $\pm$ 1.57	2-6 mg/kg	72% $\uparrow$	Exceeded
Mn	Water	19.56 $\pm$ 4.01	50-500 $\mu$ g/L	Below	Safe
	Soil	14.01 $\pm$ 5.60	300-900 mg/kg	Below	Safe
	Feathers	2.06 $\pm$ 0.53	1-5 mg/kg	Within	Normal
Cu	Water	7.43 $\pm$ 1.04	1-2 mg/L	272% $\uparrow$	Exceeded
	Soil	6.75 $\pm$ 1.16	30-100 mg/kg	Below	Safe
	Feathers	3.09 $\pm$ 0.59	2-10 mg/kg	Within	Normal
Zn	Water	0.62 $\pm$ 0.21	0.1-5 mg/L	Within	Safe
	Soil	2.34 $\pm$ 0.76	70-400 mg/kg	Below	Safe
	Feathers	7.18 $\pm$ 1.32	50-200 mg/kg	Below	Safe

**Table 3: Pearson correlation coefficients between heavy metals (Pb, Mn, Cu, and Zn) across all sample matrices (water, soil, and feathers) of cattle egret.**

	Pb	Mn	Cu	Zn
Pb	1			
Mn	-0.019	1		
Cu	-0.024	0.510*	1	
Zn	0.009	-0.524**	-0.583**	1

Correlation coefficients ( $r$ ) represent relationships between different metal concentrations, calculated by pooling data from all matrices (water, soil, feathers). \*Significant at  $p < 0.05$ ; \*\*highly significant at  $p < 0.01$  (2-tailed).

Using pooled data from all sample matrices (water, soil, and feathers), Pearson correlation coefficients were computed to investigate connections between concentrations of several heavy metals (Pb, Mn, Cu, and Zn). A substantial positive connection between Mn and Cu was found in the analysis ( $r = 0.510$ ,  $p < 0.05$ ), indicating that these metals might come from

common anthropogenic sources such irrigation inputs or agricultural runoff. On the other hand, Zn exhibited substantial negative correlations with both Cu ( $r = -0.583$ ,  $p < 0.01$ ) and Mn ( $r = -0.524$ ,  $p < 0.01$ ), suggesting distinct accumulation processes or environmental behavior. These associations show different bioavailability in different environmental compartments and intricate metal interactions.

Box-and-whisker plots for Mn, Pb, Cu, and Zn in soil, water, and feathers are shown in Figure 3. Plots of the various sample types show outliers and variability. Water and soil had much higher levels of Mn and Cu,

whereas feathers had significantly higher levels of Zn. The non-significant p-value from the ANOVA was in line

with the overlapping distributions of Pb levels.

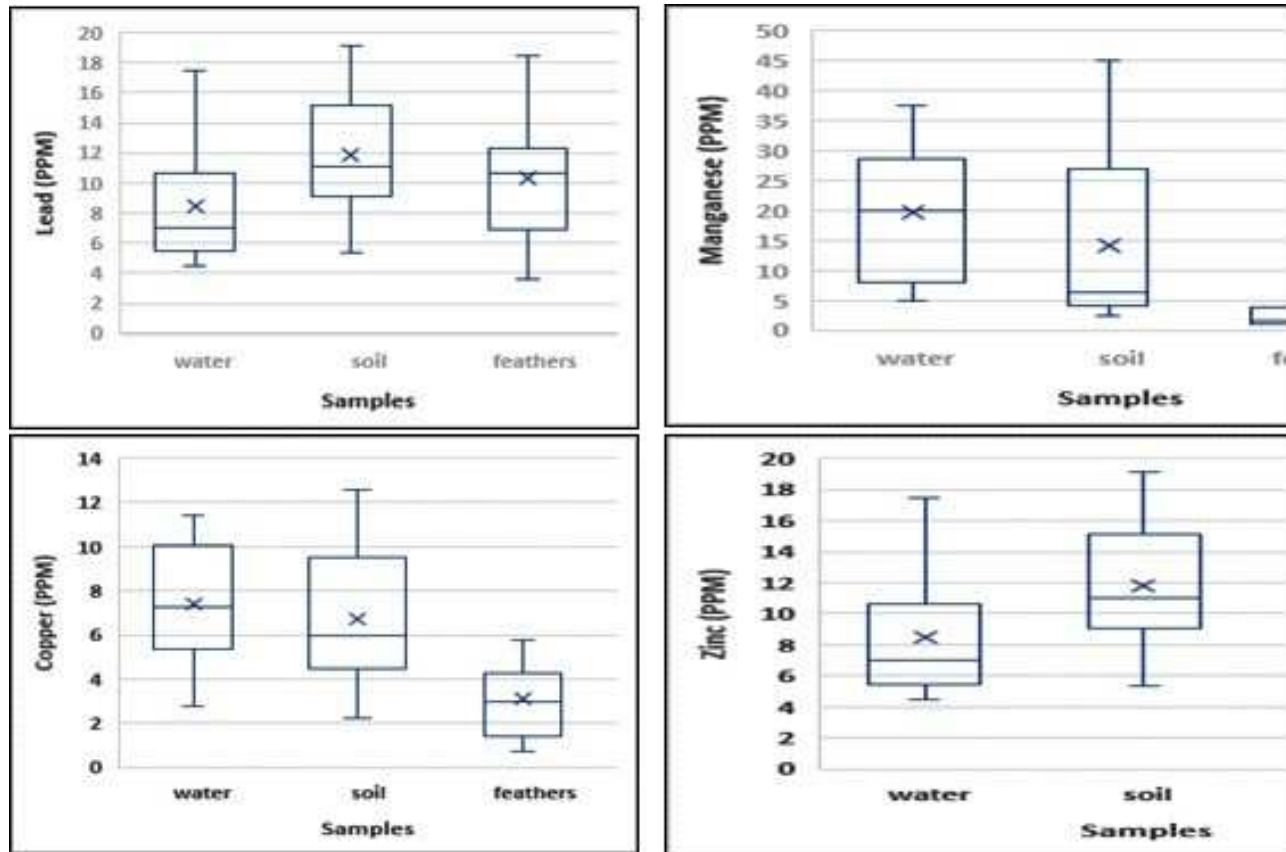


Figure 3: Box-and-whisker plots of metals in water, soil and feathers of cattle egret

## DISCUSSION

In developing places like South Asia, where untreated industrial and domestic discharges often infiltrate natural water bodies, environmental pollution—especially from heavy metals—poses a serious ecological concern (Samsonova and Kirilenko, 2024; Karn and Harada, 2001). This issue is made worse in Pakistan by the extensive use of agrochemicals and the country's fast urbanization, particularly in areas that receive irrigation from canals like Pakpattan.

Because of their place in the food chain, exposure to contaminants at different trophic levels, and physiological sensitivity to them, birds are generally acknowledged as reliable bioindicators of metal contamination (Lodenus and Solonen, 2013; Jota Baptista *et al.*, 2022). This idea is supported by our data, which showed notable variations in Mn, Cu, and Zn concentrations between soil, water, and *Bubulcus ibis* feathers.

In comparison to Malik and Zeb (2009), who found significantly greater levels of Zn (133.8 ppm) and Pb (37.5 ppm) in cattle egret feathers from more

industrialized sites, the heavy metal concentrations found in the current study were typically lower. Similarly, very high zinc levels (63.3 ppm) in *Egretta alba* were found by Honda *et al.* (1986). These discrepancies could be the result of buildup unique to a species, different sources of pollution, or variations in the sample conditions. Our soil and water samples have comparatively greater metal concentrations than those of Pandiyan *et al.* (2020) and Tan *et al.* (2021), most likely as a result of Pakpattan's confined wastewater discharge and agricultural runoff.

According to previous research (Malik and Zeb, 2009), zinc was found in the highest amounts in feathers. This suggests that zinc is bioaccumulated through food intake and metabolic affinity for keratin-binding. Zn may enter the food chain through fertilizer use and agricultural runoff, which is consistent with the elevated Zn levels. In contrast, Mn and Cu were more prevalent in soil and water, most likely as a result of contamination from irrigation and soil leaching. These results lend credence to the notion that metals' environmental partitioning varies according to their chemical characteristics and regional consumption patterns (Pandiyan *et al.*, 2020; Tan *et al.*, 2021).

Interestingly, Pb was found in all media but did not exhibit statistically significant change among matrices ( $p = 0.244$ ). This implies that there is either a large baseline concentration of lead in the environment or that there is not enough data to identify minute variations. Furthermore, Pb's extended half-life and persistence in sediments may lead to a more uniform distribution, particularly if birds are exposed in comparable polluted locations. Other investigations that found Pb exposure to be widespread but did not statistically distinguish between organs also found similar results (Sanpera *et al.*, 2000; Hernández *et al.*, 1999).

Mn and Cu showed a positive correlation, according to correlation analysis, suggesting that they came from the same or co-occurring sources, including agricultural effluents. Zn, on the other hand, demonstrated strong negative associations with both Mn and Cu, pointing to a unique biological regulation pattern or contamination process. These connections show intricate metal dynamics in the environment that are impacted by biotic absorption processes as well as human inputs.

For instance, our Zn and Pb levels were somewhat lower than those of Malik and Zeb (2009), who observed greater levels in the feathers of cattle egrets from more urbanized areas. This could be because of variations in industrial loads or modifications in agricultural methods. Our results are significantly lower than the extraordinarily high Zn levels (63.3 ppm) reported in *Egretta alba* by Honda *et al.* (1986), suggesting possible interspecies and geographical variations in metal uptake.

The feathers of *Bubulcus ibis* may be useful as a non-invasive biomonitoring method for identifying metal contamination in canal-based agro-ecosystems, according to our findings. Programs for avian-specific biomonitoring should be a part of Pakpattan conservation initiatives. Regulators ought to enforce appropriate waste management, limit the overuse of pesticides and fertilizers high in metals, and encourage safer farming methods. Future research on bioaccumulation trends would benefit from the establishment of reference locations for baseline metal levels.

This investigation validates the existence of heavy metals in soil, water, and cattle egret feathers at a few Pakpattan canal sites. The regional variation in metal concentrations that has been observed emphasizes the necessity of continuous environmental monitoring and the incorporation of bird bioindicators into public health and conservation monitoring initiatives.

**Conclusion:** The study's conclusions show that heavy metal pollution, which mostly comes from industrial processes, brick kilns, agricultural runoff, and vehicle emissions, is a real risk to Pakpattan's aquatic ecosystems. Significant amounts of Mn, Cu, and Zn were

found in soil, water, and cattle egret feathers; a high accumulation of Zn was seen in the feathers. Despite being found in all media, Pb did not differ statistically significantly between sample types. An efficient, non-invasive biomonitoring method for determining environmental contamination was the use of feathers from *Bubulcus ibis*. In order to protect local biodiversity, these findings emphasize the critical necessity for ongoing monitoring, the implementation of sustainable agriculture methods, and the enforcement of pollution control laws.

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