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Assessment of Heavy Metal Contamination in *Corvus splendens* Feathers from Contrasting Urban and Rural Environments in Punjab, Pakistan

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Rapid industrialization and urban expansion have significantly intensified the environmental contamination by heavy metals, endangering the ecological integrity and human health. This research analyzes the bioaccumulation of four heavy metals—zinc (Zn), lead (Pb), chromium (Cr), and mercury (Hg)—in the feathers of *Corvus splendens* (House Crow) across eight sites in Punjab, Pakistan. The selected sites included four urban (Madina Town, Jinnah Town, Trimun Headworks, Lahore Industrial Area) and four rural (Mailsi, Chak Jhumra, Shorkot, Jaranwala) localities, representing conflicting environmental conditions. Feather samples (n = 40) were evaluated using atomic absorption spectrophotometry to quantify metal concentrations and evaluate spatial variability. According to the results, urban sites possessed higher concentrations of all four metals compared to rural ones ($p < 0.01$). Mercury was the least accumulated metal, ranging from 0.24 to 0.76 µg/g, while zinc was the most abundant, with concentrations in Madina Town reaching 334.0 ± 9.62 µg/g. One-way ANOVA and Tukey HSD post hoc tests revealed significant inter-site inconsistency, particularly in urban areas, signifying strong associations between heavy metal accumulation and anthropogenic activity such as industrial emissions, vehicular traffic, and waste mismanagement. These findings support the suitability of *C. splendens* as a bioindicator species and highlight the efficiency of feather-based analysis as a non-invasive tool for environmental monitoring. The study emphasizes the need for strengthened pollution control measures and continued ecological surveillance in swiftly urbanizing areas of Pakistan.

Keyword: *Corvus splendens*, heavy metals, urban pollution, feather analysis, biomonitoring, zinc, lead, chromium, mercury, Punjab

Introduction

Environmental pollution poses a tenacious global challenge, menacing biodiversity, ecosystem stability, and public health. Among the most concerning pollutants are heavy metals, which are insistent, non-biodegradable, and capable of bioaccumulating in living organisms and biomagnifying through trophic chains[1]. Their increasing environmental prevalence is largely driven by anthropogenic activities such as industrial discharge, vehicular emissions, unfettered urban expansion, and the excessive use of agrochemicals [2]. In developing nations, especially in poorly regulated urban centers, these metals infiltrate both abiotic components (soil, air, water) and biotic systems, disrupting ecological balance and physiological functions across taxa[3]. It is well established that elevated levels of

essential elements such as zinc (Zn) and toxic metals like lead (Pb), chromium (Cr), and mercury (Hg) can adversely affect avian reproductive systems and induce immunotoxicity, neurotoxicity, and physiological stress[4]. Lead, a known neurotoxin, is frequently associated with sources such as batteries, paints, and fossil fuels [5]. Chromium, particularly from tanning and electroplating industries, can induce oxidative stress and nontoxicity[6]. Mercury, especially in its methylated form, bioaccumulates in both aquatic and terrestrial food webs, impairing neurological function[7]. Although zinc is an essential micronutrient, excessive exposure can result in oxidative damage and metabolic imbalances[8].

The chronic exposure of birds and other organisms to these pollutants is associated with anemia, renal dysfunction, immunosuppression, and developmental delays (UNEP, 2008). Monitoring heavy metals in urban and peri-urban environments is therefore important for understanding contaminant sources, evaluating ecological risk, and manipulative effective mitigation policies[9].

Among avian tissues, feathers are particularly ideal for non-invasive biomonitoring, as they grow, they incorporate circulating metals into their keratin matrix. Once grown, feathers also act as passive accumulators of atmospheric particulate-bound metals [10]. Birds have been documented as effective bio indicators in environmental monitoring due to their sensitivity to environmental pollutants, wide geographical distribution, and close communication with multiple ecological settings[11].

Feather-based biomonitoring has conventionally focused on raptors and waterfowl in remote or aquatic environments[12]. Moreover, urban-adapted birds such as pigeons and crows are increasingly documented as effective sentinels of urban pollution[13]. These species feed in landfills, industrial zones, and roadside environments, making them highly unprotected to anthropogenic contamination and treasured for real-time urban exposure valuation [14].

The omnivorous, highly synanthropic *Corvus splendens* (House Crow) flourishes in habitats that have been rehabilitated by humans[15]. It is seamless for spatially explicit contamination monitoring due to its year-round presence, limited home range, and ability to utilize urban resources[16]. The potential of C. splendens as a long-term environmental biomonitor is further highlighted by the fact that it has been revealed to accumulate harmful metals like Pb, Cd, and Zn in its feathers [17].

In South Asian biomonitoring studies, C. splendens is still underutilized in spite of its ecological im[18]plication[19]. Due in part to a lack of non-destructive sampling procedures and financial constraints, there is a dearth of information on avian exposure to heavy metals in Pakistan, especially in quickly emergent cities like Faisalabad[20]. The deterioration of air, water, and soil quality in these cities due to industrial growth, traffic jams, and inadequate waste management has raised questions about the long-term effects on the environment and human health[18]

This study addresses a critical knowledge gap by examining heavy metal contamination in Punjab's urban and rural environments using C. splendens feathers as a bioindicator matrix. By measuring the concentrations of four priority metals—zinc (Zn), lead (Pb), chromium (Cr), and mercury (Hg)—across eight geographically distinct sites, this study aims to assess spatial variation in metal accumulation and identify site-specific pollution hotspots. The study also evaluates the value of C. splendens as a practical and non-invasive way to monitor environmental contamination in human-dominated landscapes. The findings provide baseline information on environmental metal loads in a rapidly urbanizing region for ecological risk assessment, public health planning, and the development of targeted pollution mitigation strategies.

Materials and Methods

Study Area

This study was conducted in several urban and rural areas of Punjab, the most densely populated province in Pakistan. Punjab is situated between latitudes 27°42′N and 34°01′N and longitudes 69°20′E and 75°20′E, encompassing an area of approximately 205,344 km². From vast agricultural landscapes to rapidly developing urban centers, the province is home to a variety of ecological zones. These inconsistent land-use patterns provided a suitable environmental gradient for evaluating the spatial variation in heavy metal pollution.

Sampling Sites

Eight geographically diverse sites—four urban and four rural—were used for collecting feather samples of *Corvus splendens* (House Crow). Intense anthropogenic activities, such as waste accumulation, automobile exhaust, and industrial emissions, are present at the urban sites of Madina Town, Jinnah Town, Trimun Headworks, and Lahore Industrial Area. With relatively little

industrial pressure, the rural locations of Mailsi, Chak Jhumra, Shorkot, and Jaranwala are mostly agricultural. To ensure effective feather regeneration and sampling consistency, sampling was conducted between March and June 2023, coinciding with the breeding and molting season of C. splendens.

Sample Collection

Birds were caught using mist nets placed near feeding and roosting sites. Each biological information (sex and age class, if apparent), date, time, and GPS location was recorded. To make composite samples, feathers were meticulously removed from the breast and tail areas. Breast feathers were preferred because of the stronger physiological association between them and internal metal exposure[21, 22]. All samples were stored in sterile, sealed polyethylene bags at room temperature before processing.

Sample Preparation

Using a standardized three-step procedure, feathers were filleted to eliminate external contaminants: (1) washing with distilled water, (2) immersion in sterile deionized water, and (3) a final rinse with acetone (dimethyl ketone). For eight hours, cleaned feathers were dried at 80°C in a hot air oven [23]. A sterile ceramic mortar and pestle were used to grind the sample into a fine powder. Each sample's precise 0.5 g of powdered feather was moved to Teflon digestion vessels.

Sample Digestion

The feather powder was treated with concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) before being heated to 120°C for 24 hours. One milliliter (mL) of hydrogen peroxide (H2O2) was added in the second phase, and the mixture was allowed to stabilize at room temperature for 24 hours. For further analysis, the resultant solution was filtered and diluted to a known volume using deionized water.

Analysis of Metals

Atomic Absorption Spectrophotometry (AAS; PerkinElmer Analyst 700) was used to measure the concentrations of metals (Zn, Pb, Cr, and Hg) at the Hi-Tech Analytical Laboratory of the University of Agriculture, Faisalabad. Calibration was done using certified reference standards.

Statistical Analysis

All statistical analyses were conducted using R statistical software (version X.X.X). One-way analysis of variance (ANOVA) was used to estimate differences in heavy metal concentrations between specimen sites. Tukey's Honest Significant Difference (HSD) post hoc test was used for numerous pairwise comparisons. Additionally, the study looked into correlations between metal concentrations using Pearson correlation analysis. The threshold for statistical significance was set at $p < 0.05$.

Ethical Statement

All bird handling and sampling procedures were conducted by the ethical standards of the University of Agriculture, Faisalabad. It complied with national wildlife research guidelines. Birds were handled minimally and released unharmed after feather collection. No live animals were harmed or euthanized in the course of this study.

RESULTS

3.1. Spatial Patterns of Heavy Metal Accumulation in *Corvus splendens*

Forty feather samples of *Corvus splendens*, five individuals per site, were collected from eight locations across Punjab to measure the concentrations of four heavy metals: zinc (Zn), lead (Pb), chromium (Cr), and mercury (Hg). The study sites were separated into two groups: urban (Madina Town, Jinnah Town, Trimun Headworks, Lahore Industrial Area) and rural (Mailsi, Chak Jhumra, Shorkot, Jaranwala) in order to evaluate environmental differences in metal exposure.

All metal concentrations were consistently and significantly higher at urban sites than at rural ones ($p < 0.01$). The most common metal, zinc, was found in the highest mean concentration in Madina Town feathers (334.0 ± 9.62 µg/g). Lead and chromium were abundant, while mercury had the lowest total concentrations but still showed significant urban enrichment. Rural regions, particularly Shorkot and Chak Jhumra, had the lowest mean concentrations of all four metals.

The mean ± standard deviation of metal concentrations by site is shown in Table 1. The two urban areas with the highest burdens were Madina Town and Lahore Industrial Area. Madina Town had the highest levels of Zn (334.0 µg/g) and Pb (13.0 µg/g), while Lahore Industrial Area had the highest levels of Cr (6.2 µg/g) and Hg (0.66 µg/g). On the other hand, Hg concentrations in rural areas stayed below 0.30 µg/g, while Zn and Cr concentrations were lowest in Shorkot (137.0 and 1.7 µg/g, respectively). These trends point to site-specific human influences on C. splendens metal exposure, which are probably related to industrial activity, land use variation, and traffic emissions.

Table 1. Mean ± standard deviation (SD) of heavy metal concentrations (µg/g dry weight) in feathers of *Corvus splendens* from urban and rural sites in Punjab, Pakistan (n = 5 per site).

Site	Type	Zn (µg/g)	Pb (µg/g)	Cr (µg/g)	Hg (µg/g)
Madina Town	Urban	334.0 ± 9.62	13.0 ± 0.37	7.2 ± 0.27	0.76 ± 0.05
Jinnah Town	Urban	290.0 ± 7.91	10.0 ± 0.37	5.8 ± 0.14	0.56 ± 0.05
Trimun Headworks	Urban	260.0 ± 7.91	8.9 ± 0.28	4.8 ± 0.19	0.44 ± 0.05
Lahore Industrial Area	Urban	320.0 ± 7.91	11.4 ± 0.30	6.2 ± 0.15	0.66 ± 0.05
Mailsi	Rural	152.0 ± 6.78	4.8 ± 0.19	3.0 ± 0.15	0.30 ± 0.05
Chak Jhumra	Rural	172.0 ± 3.67	5.3 ± 0.19	2.2 ± 0.11	0.24 ± 0.05
Shorkot	Rural	137.0 ± 3.80	4.0 ± 0.16	1.7 ± 0.11	0.26 ± 0.05
Jaranwala	Rural	165.0 ± 3.81	6.2 ± 0.11	2.7 ± 0.11	0.28 ± 0.05

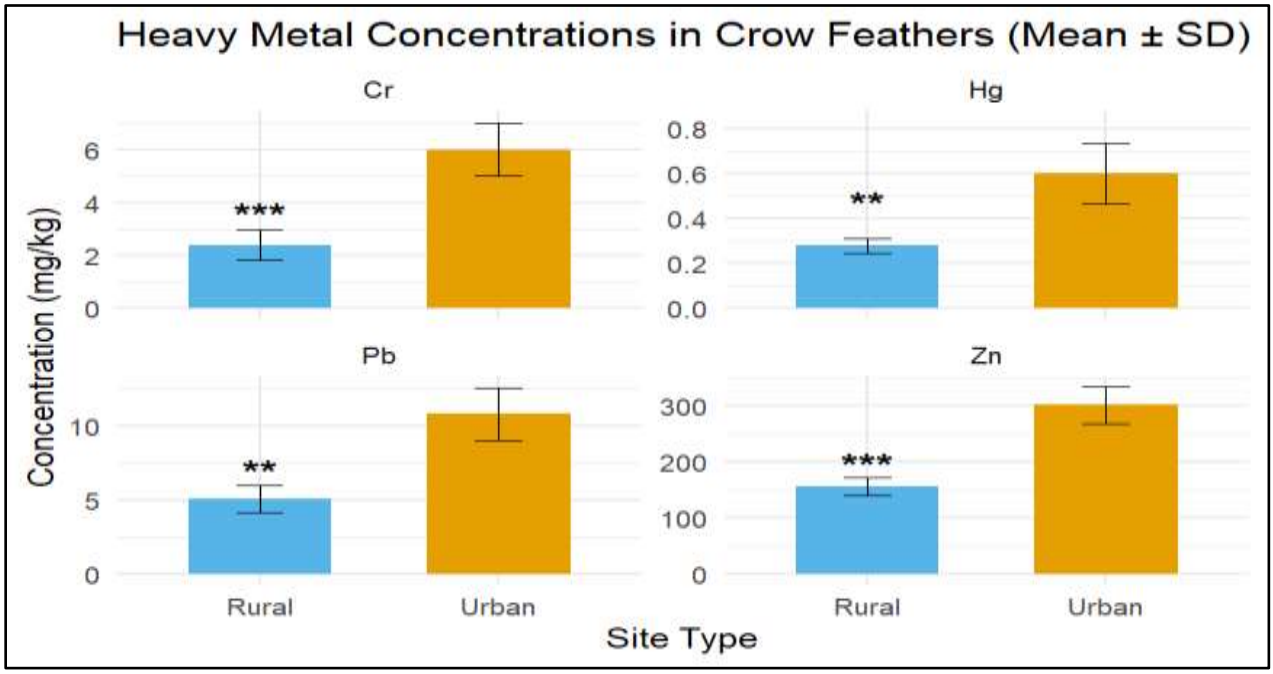

Figure 1. Mean concentrations (µg/g dry weight) of Zn, Pb, Cr, and Hg in feathers of *Corvus splendens* collected from eight urban and rural sites across Punjab. Error bars represent ± standard deviation. Urban sites consistently showed higher concentrations across all metals.

Inter-Site Differences Based on Tukey HSD Post Hoc Analysis

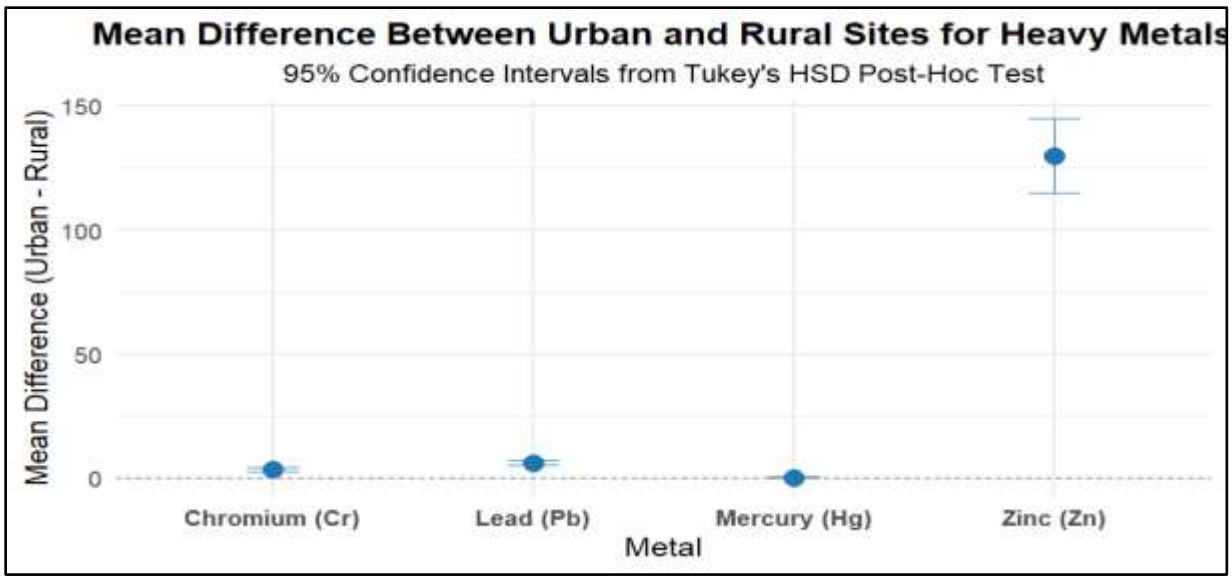

Figure 2. Tukey HSD post hoc comparisons for Zn, Pb, Cr, and Hg concentrations across study sites. Horizontal bars represent 95% confidence intervals; comparisons that do not overlap zero are statistically significant at $p < 0.05$.

Table S2. Summary of one-way ANOVA comparing heavy metal concentrations in feathers of *Corvus splendens* between urban and rural sites in Punjab, Pakistan.

Metal	**F-value**	**p-value**	**Significance**
Zinc (Zn)	62.36	0.000219	*** (p < 0.001)
Lead (Pb)	33.08	0.0012	** (p < 0.01)
Chromium (Cr)	39.47	0.000757	*** (p < 0.001)
Mercury (Hg)	21.33	0.00362	** (p < 0.01)

*Significance codes: ***p < 0.001, **p < 0.01, *p < 0.05*

Table S3. Tukey HSD post hoc comparisons of heavy metal concentrations (µg/g) in feathers of *Corvus splendens* between urban and rural sites of Punjab, Pakistan. Bold comparisons represent statistically significant differences (p < 0.05).

Metal	**Comparison**	**Mean Diff**	**Lower CI**	**Upper CI**	**p-adj**	**Sig.**
Zinc	Madina Town – Mailsi	182.00	167.90	196.10	<0.001	***
	Lahore Ind. Area – Mailsi	168.00	153.90	182.10	<0.001	***
	Jinnah Town – Mailsi	138.00	123.90	152.10	<0.001	***
	Trimun Headworks – Mailsi	108.00	93.90	122.10	<0.001	***
	Shorkot – Mailsi	-17.00	-31.10	-2.90	0.0096	**
	Jaranwala – Mailsi	13.00	-1.10	27.10	0.0883	ns
	Chak Jhumra – Mailsi	20.00	5.90	34.10	0.0015	**

Lead	Madina Town – Mailsi	8.24	7.69	8.79	<0.001	***
	Lahore Ind. Area – Mailsi	6.62	6.07	7.17	<0.001	***
	Jinnah Town – Mailsi	5.28	4.73	5.83	<0.001	***
	Trimun Headworks – Mailsi	4.14	3.59	4.69	<0.001	***
	Shorkot – Mailsi	-0.74	-1.29	-0.19	0.0026	**
	Jaranwala – Mailsi	1.44	0.89	1.99	<0.001	***
Chromium	Madina Town – Mailsi	4.14	3.79	4.49	<0.001	***
	Lahore Ind. Area – Mailsi	3.18	2.83	3.53	<0.001	***
	Jinnah Town – Mailsi	2.76	2.41	3.11	<0.001	***
	Trimun Headworks – Mailsi	1.76	1.41	2.11	<0.001	***
	Shorkot – Mailsi	-1.36	-1.71	-1.01	<0.001	***
	Chak Jhumra – Mailsi	-0.86	-1.21	-0.51	<0.001	***
	Jaranwala – Mailsi	-0.36	-0.71	-0.01	0.040	*
Mercury	Madina Town – Mailsi	0.44	0.335	0.545	<0.001	***
	Lahore Ind. Area – Mailsi	0.32	0.215	0.425	<0.001	***
	Jinnah Town – Mailsi	0.24	0.135	0.345	<0.001	***
	Trimun Headworks – Mailsi	0.12	0.015	0.225	0.016	*
	Shorkot – Mailsi	-0.04	-0.145	0.065	0.915	ns
	Jaranwala – Mailsi	-0.04	-0.145	0.065	0.915	ns
	Chak Jhumra – Mailsi	-0.08	-0.185	0.025	0.245	ns

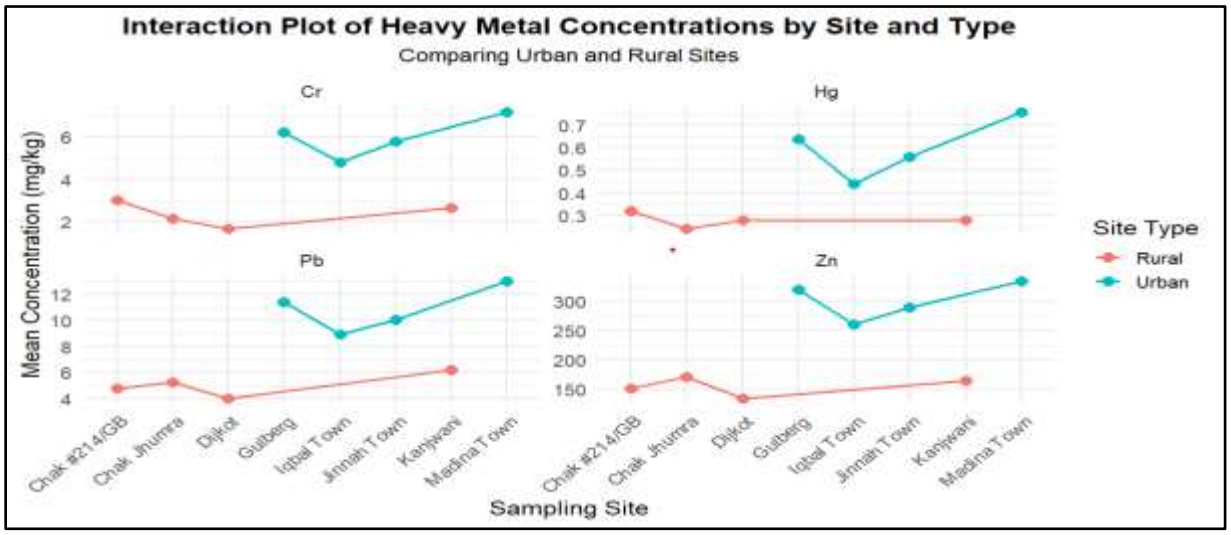

Figure 3. Interaction plot showing variation in heavy metal concentrations by site and site type (urban vs. rural). Steeper slopes in urban locations indicate stronger site effects, particularly for Zn and Cr.

Interaction plots provided additional insight into the concentration trends across sites and region types. Significant side effects from industrial activity and anthropogenic pressure were indicated by the steeper gradients and higher overall values found in urban areas, particularly for Zn and Cr. These visuals demonstrate how metal exposure in cities differs by location and support the statistical findings.

3.2. Statistical Analysis of Urban–Rural Differences

One-way Analysis of Variance (ANOVA) was done by using R, skimpy significant differences ($p < 0.01$)

in heavy metal concentrations concerning urban and rural environments for all four metals. Urban sites constantly recorded higher mean values. The largest dissimilarity was observed for zinc ($F = 62.36$, $p < 0.001$), followed by chromium ($F = 39.47$, $p < 0.001$), lead ($F = 33.08$, $p < 0.01$), and mercury ($F = 21.33$, $p < 0.01$). These results, detailed in Supplementary Table S2, sanction significant spatial heterogeneity likely associated with varying degrees of human impact.

A Tukey HSD post hoc test was used to evaluate pairwise differences across all eight sites after an ANOVA. The findings show that metal concentrations vary significantly, especially between urban and rural locations. In contrast to all rural sites and even some other urban areas, Madina Town and the Lahore Industrial Area showed noticeably higher concentrations of the majority of metals.
For example, despite both being urban areas, the levels of zinc in Madina Town were significantly higher ($p < 0.001$) than in Trimun Headworks, highlighting localized variations in exposure sources. While all rural values remained significantly lower than their urban counterparts ($p < 0.05$), Mailsi and Chak Jhumra recorded soberly higher concentrations than Shorkot and Jaranwala.

DISCUSSION

The concentrations of zinc (Zn), lead (Pb), chromium (Cr), and mercury (Hg) in *Corvus splendens* feathers collected from urban and rural environments in Punjab, Pakistan, are thoroughly evaluated in this study. The results show that urban areas have noticeably higher metal burdens. This trend aligns with the established connection between urbanization and increased exposure to environmental metals as a result of industrial discharge, waste incineration, vehicle emissions, and inadequate waste management techniques. Similar patterns have been seen in South Asian cities like Tiruchirappalli, India [24], Dhaka, Bangladesh [25], and Colombo, Sri Lanka[26] where birds living in populated and industrialized areas carry significantly higher metal loads than their rural counterparts.
Our urban house crows possessed higher levels of zinc than rural crows in India[27] , and they were similar to concentrations in Passer domesticus in tandem a pollution gradient in Morocco [28]. Car tire abrasion, galvanized metal corrosion, and consumption of tainted human food waste—all of which are easily accessible to this synanthropic species—are probably the causes of these high Zn levels[29]. Although zinc is a necessary micronutrient, too much of it can affect enzymatic and metabolic functions[30]. While the high zinc concentrations observed here are below the lethal thresholds documented for the liver and kidney tissues of birds[31], they nevertheless raise questions about possible sublethal effects, especially when combined pollutants are present.
Various urban locations, especially Jinnah Town and Lahore Industrial Area, had Lead concentrations above the eco-toxicological threshold of 9 mg/kg, which has been shown to impair *Turdus merula* reproduction [32]. Similar or higher Pb loads have been reported for house crows in Malaysian industrial areas[33] and urban ravens in Israel[34]. The main causes of these Pb loads are industrial dust, informal battery recycling, and atmospheric deposition from leaded gasoline legacy. Exogenous installation on feathers is a major pathway, although some Pb may come from feed [35, 36]
Urban chromium levels were comparable to those observed in crows residing in Indian tannery areas[37] and advanced toxicity is close to reproductive depreciation reported in seabirds[38]. The strong industrial base of Lahore, predominantly in leather tanning, electroplating, and pigment manufacturing, is likely a chief source of Cr contamination, sparkly patterns also observed in urban passerines in China[39] and South America[40]
Mercury concentrations, even though the lowest among the metals studied, were considerably higher in urban birds than rural equivalents, in agreement with findings from Colombo[33] and littoral Chinese cities [39]Hg sources in urban Pakistan may comprise coal ignition, open waste burning, and industrial emissions. Even at sublethal concentrations, Hg can source endocrine disruption, behavioral changes, and neurological injuries in birds [41].
Evaluations with local literature specify that Zn and Pb levels in our urban birds are comparable with those described in House Crows from Faisalabad and Lahore[42, 43] but surpass levels in Common Mynas (*Acridotheres tristis*) and House Sparrows (*Passer domesticus*) from related environments[44, 45]. This difference may be clarified by species-specific variations in foraging range, dietary flexibility, and feather melanin chemistry, which influence metal-binding capacity [46, 47]. Worldwide our Pb and Zn concentrations are comparable to or higher than those in urban passerines in Malaysia [48], India [24] and Bangladesh [25]. Generally above values noted in

European passerines [49, 50], likely reflecting differences in industrial emission regulations and urban waste management standards.

The observed bioaccumulation patterns demonstrate efficient absorption from environmental sources such as contaminated prey, trash, and inhaled particulates, with bioaccumulation factors (BAF) for zinc and lead in urban birds greater than one. The risk of exposure is increased by the House Crow's omnivorous diet and proximity to human wastewater channels. Our results corroborate previous studies that discovered urban scavengers gather more pollutants than specialized feeders[51].

The present study points out important spatial tendencies, but the lack of concurrent environmental sampling limits decisive source ascription. Future studies should assimilate soil, water, and air specimens from the same sites, integrate seasonal monitoring, and apply metal speciation and isotopic scrutinizes to differentiate between industrial and geogenic sources. Furthermore, various bird species in such assessments would also provide insight into trophic-level differences in metal exposure.

From a management perspective, the elevated Zn, Pb, and Cr burdens observed in urban *C. splendens* not only signal risks to urban avian biodiversity but also have potential implications for human health, given the species' close contact with anthropogenic food sources. Mitigation strategies should prioritize sterner industrial effluent controls, lessening of Pb and Cr emissions from vehicular and industrial sources, and the amalgamation of non-invasive biomonitoring methods, such as feather analysis, into urban conservation health surveillance. Such measures would contribute to achieving international ecological and public health targets, as well as those under the Sustainable Development Goals.

CONCLUSION

This study suggests that *Corvus splendens* feathers provide a practical, non-invasive way to monitor heavy metal pollution in Punjab, Pakistan, both in rural and urban areas. Urban areas have consistently higher levels of Zn, Pb, Cr, and Hg, indicating the significant impact of human activities on environmental metal loading, particularly vehicle traffic, industrial emissions, and inadequate waste management. Zn was the most abundant element, whereas Hg had the lowest concentration but still showed significant urban enrichment. Given that long-term exposure to sublethal levels of Pb, Cr, and Hg can affect avian reproduction, immunity, and survival and may indicate hazards to other organisms, including humans, these findings have significant ecological and public health ramifications. In order to reduce pollutant sources, the baseline data presented here can be used to inform urban planning, enhance environmental regulations, and direct targeted mitigation strategies. Our capacity to monitor contamination trends and safeguard ecosystems and human health in quickly urbanizing settings will be further improved by extending such avian biomonitoring techniques to other areas and incorporating biochemical and isotopic analyses.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

G. Javaid conceived the idea, wrote the initial draft of the manuscript, and handled the correspondence, serving as the main contributor to the study. A. Fatima contributed to the study design, performed data analysis, and assisted in manuscript preparation. A. Sarwar and F. Hassan made corrections and refined the content, while A. Razaq and N. Zahra proofread and improved specific sections of the manuscript. All authors read and approved the final version of the manuscript.

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