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Monitoring of heavy metal pollution in urban and rural environments across Pakistan using House crows (*Corvus splendens*) as bioindicator

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Abstract A widely distributed urban bird, the house crow (*Corvus splendens*), was used to assess bioavailable heavy metals in urban and rural environments across Pakistan. Bioaccumulation of arsenic (As), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe), manganese (Mn), chromium (Cr), and copper (Cu) was investigated in wing feathers of 96 crows collected from eight locations and categorized into four groups pertaining to their geographical and environmental similarities. Results revealed that the concentrations of Pb, Ni, Mn, Cu, and Cr were positively correlated and varied significantly among the four groups. Zn, Fe, Cr, and Cu regarded as industrial outputs, were observed in birds both in industrialized cities and in adjoining rural agricultural areas irrigated through the Indus Basin Irrigation System. Birds in both urban regions accrued Pb more than the metal toxicity thresholds for birds. The house crow was ranked in the middle on the metal accumulation levels in feathers between highly accumulating raptor and piscivore and less contaminated insectivore and granivore species in the studied areas,. This study suggests that the house crow is an efficient bioindicator and supports the feasibility of using feathers to discriminate the local pollution differences among terrestrial environments having different levels and kinds of anthropogenic activities.

Keywords Anthropogenic · Birds · Bioindicator · Heavy metals · Passerine · Principal component analysis · Urban

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Introduction

Assessments of heavy metal pollutants are one of the major pressing environmental challenges with significant health implications for both human and other biological communities (Krishna et al., 2017). Heavy metals are non-biodegradable and bioaccumulate in the food chain if their uptake is increased due to dietary and habitat modifications (Klaassen & Amdur, 2013). Therefore, in the past laboratory toxicological techniques to assess ecosystems were criticized to poorly reflect the real damage that pollutants are capable of causing in living organisms, as the metals can concentrate in living organisms. Therefore, bioindicator species and biomonitoring are considered useful methodologies for both qualitative and quantitative measurements of the effects of pollution on living organisms in the real world.

Pakistan has undergone a vast scale of industrialization over the past 70 years. Unregulated industrial waste disposal, along with unplanned urbanization and deforestation, has increased anthropogenic pollution to a hazardous level and degraded all components of the natural environment (Hamid et al., 2018; Von Schneidemesser et al., 2010). Tons of untreated sewage, solid sludge, and industrial effluents containing alkalis, acids, salts, and heavy metals are drained daily into seasonal streams that eventually flow into the rivers (Saleem, 2017). In addition, waste dumping on open land and wetlands and agricultural runoff are deteriorating the air, soil, and water ecosystems and food chains (Batool et al., 2019). Many ecotoxicological studies have reported high loads of heavy metals in air, surface or groundwater, soil sediments, and biota including fish and birds and a variety of foods (Boncompagni, 2003; Farooqi et al., 2007; Nawaz et al., 2010; Khan, 2013b; Waseem et al., 2014; Ansari et al., 2018; Sarfraz et al., 2018; Ur Rehman et al., 2018). Limited available data on human biomonitoring also presented a comparatively high value of most of the toxic metals in biological fluids/tissue in the general population (Waseem & Arshad, 2016).

In the past two decades, several studies were conducted using birds to evaluate heavy metal and organic pollution in Pakistan. These screened a limited number of replicates of diverse species (Abbasi et al., 2015a; Abbasi et al., 2015b; Abbasi et al., 2016) or used top predator species that may have concentrated the pollutants from niche environments (Movalli, 2000; Boncompagni et al., 2003; Malik & Zeb, 2009; Nighat et al., 2013; Ullah et al., 2014; Abdullah et al., 2015; Abbasi et al., 2017a; Abbasi et al., 2017b; Kanwal et al., 2020). Heavy metals have differential patterns of bioaccumulation across tissue types, and a wide variety of sampling techniques are used based on study accumulation in internal body organs like the kidney and the liver (Horai et al., 2007; Kojadinovic et al., 2007), blood (Kanwal et al., 2020), eggshells (Burger & Gochfeld, 2007), feathers (Metcheva et al., 2006), or prey samples of bird (Ullah et al. 2014; Finger et al., 2017). Feathers, in particular, provide a nondestructive and easily accessible means of monitoring tool (Burger & Burger, 1993; Jaspers et al., 2009; Abbasi et al., 2016). Certain bird species have been found to be useful reliable bioindicators for heavy metal pollution in the air, land, or water (Malik & Zeb, 2009).

In this study, we used an urban bird species, the common house crow (*Corvus splendens*), to assess variations in heavy metal bioaccumulation in various urban and rural settings in Pakistan. The house crow is widely distributed in South Asia, including Pakistan, but has also migrated to a number of countries further away (Krzemińska, 2016). It is a sedentary omnivorous species that live in close proximity to human settlements and feeds on human food wastes, small animals, eggs, nestlings, and animal carcasses. Our aims were to investigate the variation in metal accumulation in the house crows from different urban and rural territories across Pakistan and determine the gender-biased differences, if any, as the female birds regularly shed a considerable load of heavy metals in the eggshell (Hashmi et al., 2015). Our objectives for this study were to (1) determine the distribution and contamination levels of heavy metals and metalloids in house crows from different urban and rural settings across the country; (2) investigate any inter-metal correlations and their sources in the studied locations; (3) compare the metal accumulation levels in house crows with reports from other birds species that share the same habitat and with the metal toxicity thresholds determined for birds; and (4) investigate any gender-biased metal deposition among male and female birds.

Materials and methods

Study area and feather collection

Fully grown wing and tail feathers were collected from 96 individual house crows in eight locations across three provinces, Punjab, Sindh, and Khyber Pakhtunkhwa in Pakistan (Fig. 1; Table 1). Samples were taken from adult birds culled by municipal authorities or local farmers in December 2016 and then again in January 2018. The culling was undertaken to protect the crops and to eradicate the overpopulation of house crows in the cities.

Samples collected from the eight various locations in the northern or southern region of Pakistan exhibiting urban or rural environmental conditions were pooled into four groups for the comparative analysis: south urban, south rural, north urban, and north rural.

(a) South urban: Samples collected from the country's largest metropolitan and industrial port city, Karachi (24° 56′ N, 67° 07′ E) represent urban anthropogenic environment.

(b) South rural: Jacobabad (28° 16′ N, 68° 24′ E) is a rural agricultural district in the southern province, Sindh. Jacobabad, has a well-structured irrigation system of canals originating from the Indus River. The local livelihood mainly relies on agriculture, and only agro-based industries including husk and flour mills are present in the district.

(b) North urban: In the north of country, Punjab is the most urbanized province having intensive industrial and agricultural activities. Urban sampling was conducted in Lahore (31° 31′ N, 74° 13′ E) and Multan (30° 14′ N, 71° 25′ E). Lahore is the provincial capital and the second most populated city in the country with major industrial infrastructure. Multan is located in the highly fertile alluvial Punjab plain and in the past few decades agro-based industries, such as fertilizer plants, paper, dye, textile, and sugar factories have started in the city and imposed a great burden of pollution in the region (Bilal, 2018).

(d) North rural: Samples from Okara (30° 48′ N, 73° 23′ E), Gujar Khan district (33° 13′ N, 73° 10′ E), and Soon Valley (32° 37′ N, 72° 14′ E) in the Punjab province represent the rural environments. These areas have a variable scale of local economies driven by agriculture, cattle farming, and mining and do not have any substantial industrial operations. In Khyber Pakhtunkhwa, the house crow's feathers were collected from Swat Valley (34° 44′ N, 72° 17′ E), a mountainous terrain in northwestern Pakistan, where fruit farming, marble and gemstone mining, and tourism are the three primary resources for the local economy.

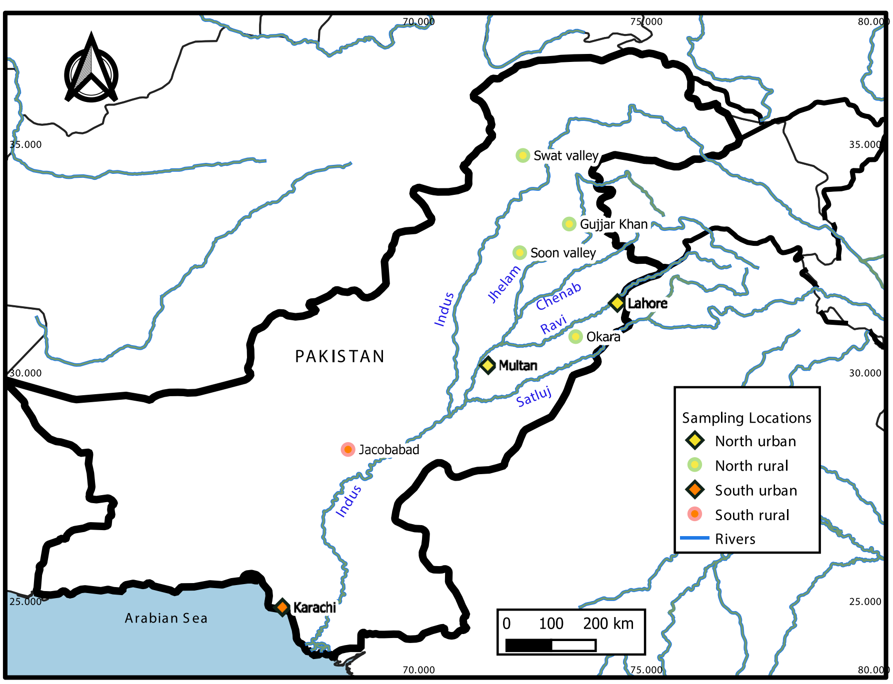

Fig. 1 Map showing sampling locations in Pakistan

Table 1 Metal concentrations ((mean ± SD) (min–max), mg/kg dw) in feathers of house crows collected from urban and rural environments of Pakistan

Region	Location	As	Zn	Pb	Cd	Ni	Fe	Mn	Cr	Cu
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
		Min–max	Min–max	Min–max	Min–max	Min–max	Min–max	Min–max	Min–max	Min–max
North urban	Lahore	0.02±0.06	247.96±95.67	19.11±9.45	0.55±0.20	0.63±1.31	185.84±66.00	16.11±5.86	0.69±1.55	6.61±1.57
	N =18	0–0.2	129–491.1	5.9–38.4	0.2–0.9	0–5.1	105.2–340.2	6.9–29.7	0–6.6	4.1–11.2
	Multan	0.23±0.30	108.17±13.07	22.86±8.33	0.15±0.08	0.71±1.42	229.26±150.16	12.47±5.84	0.83±1.05	4.08±3.35
	N =19	0.0–1.1	85.4–140.2	10.4–38.2	0.0–0.3	0.0–5.9	77.4–587.9	6.0–25.4	0.0–3.1	0.0–9.1
Overall means		0.13±0.24	174.38±96.42	21.08±8.96	0.34±0.25	0.65±1.35	208.69±118.59	14.19±6.06	0.76±1.29	5.28±2.92
North rural	Okara	0.23±0.29	239.47±78.18	12.90±6.12	0.41±0.22	2.08±2.90	155.47±42.09	17.34±5.05	2.20±3.11	6.64±1.67
	N =10	0–0.8	133.5–378.9	5.3–22.9	0.2–0.9	0.0–9.2	77.7–237.9	10.5–28.9	0.0–9.5	4.4–9.4
	Gujar Khan	ND	159.00±42.14	11.55±6.86	0.20±0.14	1.35±1.06	124.25±44.34	13.75±2.76	1.50±0.71	10.15±4.17
	N = 2		129.2–188.8	6.7–16.4	0.1–0.3	0.6–2.1	92.9–155.6	11.8–15.7	1.0–2.0	7.2–13.1
	Soon Valley	ND	113.97±28.31	3.37±3.49	0.13±0.23	0.30±0.44	211.87±267.86	9.70±7.73	0.37±0.64	9.03±6.21
	N = 3		94.9–146.5	1.3–7.4	0.0–0.4	0.0–0.8	51.6–521.1	4.6–18.6	0.0–1.1	5.3–16.2
	Swat	ND	89.54±6.57	6.25±6.62	0.00±0.00	1.39±1.76	158.33±53.35	5.89±2.02	1.62±1.82	6.59±1.18
	N = 10		82.1–99.3	1.2–17.2	0.0–0.0	0.0–5.5	86.1–248.5	3.3–8.5	0.1–5.5	5.0–8.4
Overall means		0.09±0.21	158±86.08	8.99±6.89	0.20±0.24	1.53±2.17	160.88±90.80	11.56±6.67	1.69±2.30	7.19±2.63
South urban	Karachi	ND	184.84±45.07	20.34±14.39	0.21±0.08	1.17±1.45	220.53±90.05	8.77±2.87	1.49±2.14	11.76±2.85
	N =14		117.1–298.0	5.4–54.0	0.1–0.3	0.2–5.5	79.4–450.1	3.1–12.5	0.2–8.7	7.3–19.5
South rural	Jacobabad	0.31±0.72	162.30±51.79	14.76±9.55	0.25±0.14	4.69±13.65	442.64±227.18	20.35±7.87	7.20±25.93	4.24±2.80
	N =19	0.0–2.6	112.3–324.8	6.7–40.9	0.1–0.5	0.0–53.0	58.8–972.8	5.8–35.3	0.0–112.5	0.0–9.4

Metal analysis

Feathers were washed with deionized water and acetone to remove exogenous contaminants. After washing, the feathers were oven-dried at 60 °C for 24 h and cut into 1–2-mm pieces with stainless steel scissors (Abdullah et al., 2015). For digestion, HNO_3 (69% v/v, 5 mL) (Merck Germany) was added to 0.5 g of feathers. The samples were then placed in a dry block digestor (FOSS™ Kjeldahl Digestion System) at 40 °C for 1 h. This was followed by the addition of H_2O_2 (32% v/v, 1 mL). The temperature was subsequently raised to 140 °C for another 3 h. The digested samples were then diluted with ultrapure water to bring the final volume to 50 ml and filtered with Whatman filter paper No. 1 (Janaydeh et al., 2016). The samples were stored in clean polyethylene tubes at 4 °C until measurements were carried out. The concentrations of nine trace metals arsenic (As), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe), manganese (Mn), chromium (Cr), and copper (Cu) were measured on the Perkin Elmer Optima 8000 ICP-OES.

Quality control

The quality control (QC) sample was prepared by digesting feathers of farm chickens and spiked with a known concentration (0.05 ppm) of metal standards. Samples were analyzed in batches and run in triplicate on ICP-OES, where every batch included one reagent blank and QC sample. The precision of the analytical procedures was expressed as relative standard deviation (RSD) that was calculated by dividing the standard deviation with the mean. The measurement of the process was checked using reference material, DOLT-5 (Dogfish liver), provided by the National Research Council of Canada Institute for National Measurement. The mean recoveries of the certified samples were within 15% of the certified values. The QC sample was inserted in between every 15 samples to assess the instrumental stability. The percentage metal recovery of the QCs ranged from 95 to 105%. Calibration curves were plotted using various concentrations of standard solutions (i.e., 0.01 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 1 ppm, 2 ppm, and 5 ppm). Limit of detection (LOD) was set at 3 times the standard deviation of the procedural blank (Addis & Abebaw, 2017). LOD was 0.02 mg/kg for Cd and Mn, 0.05 mg/kg for Zn and Ni, 0.1 mg/kg for Fe, Cr, and Cu, 0.2 mg/kg for Pb and 0.3 mg/kg for As. All glassware used in this study were sterilized with 10% HNO_3 to avoid possible contamination and then washed with deionized water and oven-dried before use.

Statistical analysis

Statistical analysis was conducted through SPSS version 23 with the exception of multivariate analysis that was analyzed with MVSP v3.22. The fitness to the normal distribution of the data was tested by the Shapiro-Wilk one-sample test, and the null hypothesis was rejected at $p < 0.05$. Therefore, non-parametric tests were used for further analysis. To investigate the significance of metal concentration differences among four habitats, the Kruskal-Wallis H test was applied with pairwise comparison and Bonferroni error correction at a confidence interval of 95%. Spearman's correlation coefficient analysis was used to find associations among the observed metals in individual habitat. Hierarchal cluster analysis (HACA) was performed on combined data from all collection sites to identify closely correlating groups of metals. Unweighted pair group average (UPGMA) was used for the linkage method along with Euclidean distance as the distance matrix. Principal component analysis (PCA) was performed to investigate possible sources of heavy metals in each habitat. Factor analysis was conducted using Varimax rotation with Kaiser's rule extraction. PCA biplot was developed for the principal components (PCs) extracted for eigenvalues > 1. The concentrations values of different metals varied greatly; therefore, the data was log-transformed for the HACA and PCA analysis. The Mann-Whitney U test was used to determine the significance of the difference between male and female birds to accumulate metals. The bird gender was determined using diagnostic sex-specific molecular markers.

Results and discussion

Statistical characteristics of nine heavy metals measured in 96 house crows collected from 8 locations are summarized in Table 1 and Fig. 2. No significant sex-based differences were found among birds at any of the sites. We compared our results with the available

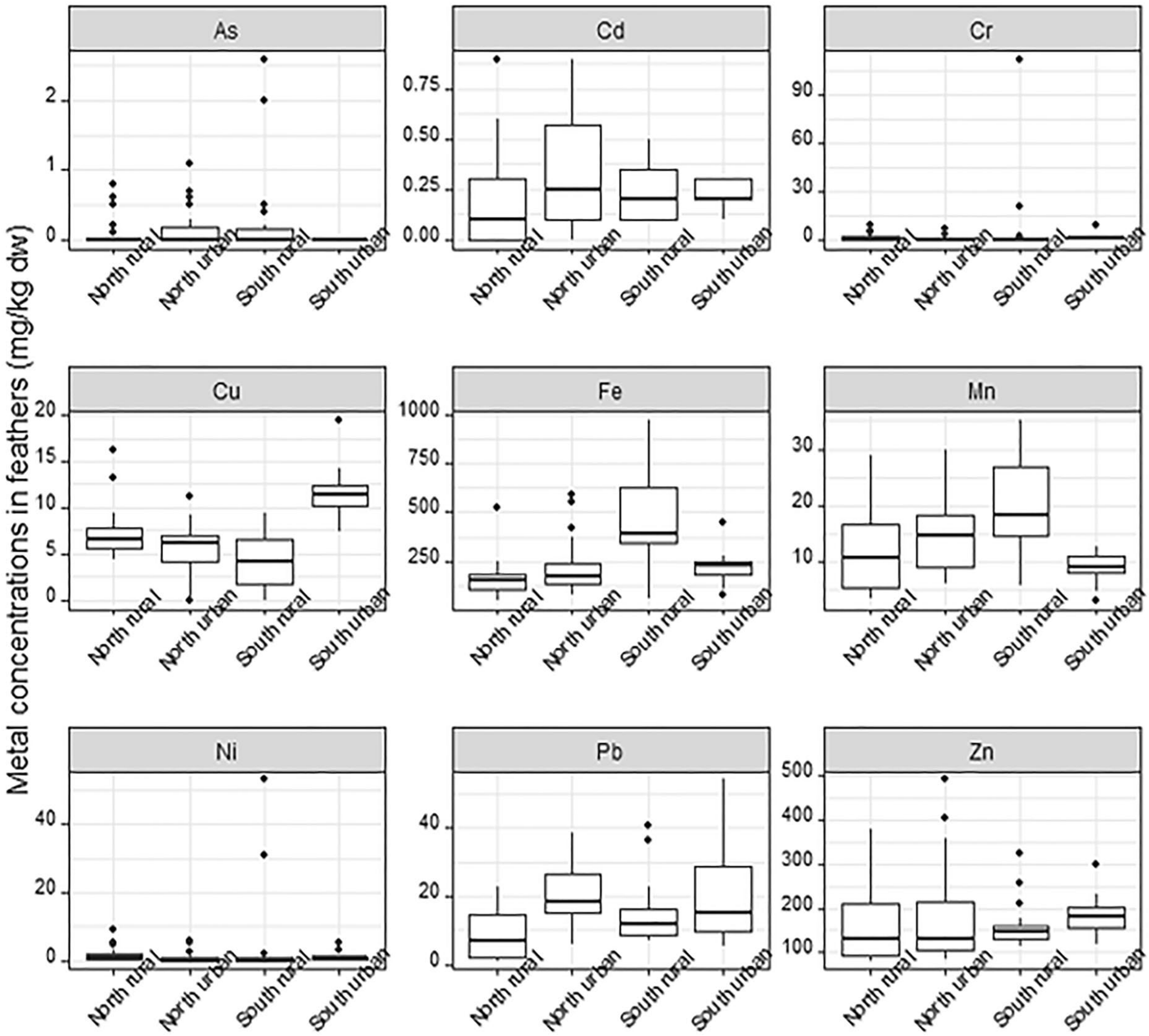

Fig. 2 Level of individual metal concentrations (mg/kg dw) in house crow feathers in urban and rural environments in the north and south part of Pakistan

literature on metal concentrations in the terrestrial bird feathers from our studied or nearby areas within Pakistan (Table 2). A comparison of heavy metal accumulation in the house crow with other crow species (*Corvidae*) reported elsewhere in the world is provided in the supplementary material (Table S1).

Metal accumulation in house crow feathers

In this study, As was only detected in the urban cities and rural sites birds from Punjab and Sindh located in the Indus River plains (Table 1). As is a water-soluble metalloid and can easily contaminate the surface and subsurface water bodies and appear in sediments. Therefore, higher levels of As have been reported in an aquatic bird, the cattle egret (21.4 mg/kg), in Lahore compared to terrestrial birds (Abdullah et al., 2015). The areas highlighted in our study with detectable As levels in house crow feathers are known to have in As-contaminated aquifers (Podgorski et al., 2017), and symptoms of arsenic toxicity are also visible in the human population in those areas (Wadhwa, 2011; Sanjrani et al., 2017.

Table 2 Comparison of mean heavy metal concentration (mg/kg dw) in house crow from this study and terrestrial birds reported from similar collection areas in Pakistan using birds' feathers

Location	Species	Order	As	Zn	Pb	Cd	Ni	Fe	Mn	Cr	Cu	References
Lahore	House crow	Passeriformes	0.02	248	19.11	0.55	0.63	185.8	16.11	0.69	6.61	This study
	Yellow wagtail	Passeriformes	-	94.15	1.87	1.14	1.52	81.93	1.8	2.28	3.77	Abbasi et al. (2015a)
	Common myna	Passeriformes	-	75.94	1.55	0.57	1.83	100.4	1.47	0.85	1.61	
Central Punjab	Steppe eagle, black kite, black-shouldered kite, common buzzard, white-eyed buzzard, marsh harrier, Eurasian sparrowhawk, red-headed vulture, shikra	Accipitriformes	14.41	116.5	28.06	5.9	79.28	-	-	-	12.77	Nighat et al. (2013)
	falcon, red-headed merlin, common kestrel	Falconiformes	5.13	201.5	19.18	3.69	148.3	-	-	-	32.91	Nighat et al. (2013)
	Short-eared owl, spotted owlet, eagle owl, barred owlet	Passeriformes	4.72	60.96	12.83	0.88	55.99	-	-	-	5.59	Nighat et al. (2013)
Okara	House crow	Passeriformes	0.23	239.5	12.9	0.41	2.08	155.5	17.34	2.2	6.64	This study
	Shikra	Accipitriformes	-	122	2.68	1.01	2.25	111.4	2.72	2.59	3.49	Abbasi et al. (2015a)
	little owl	Passeriformes	-	100.8	2.68	0.87	1.2	100.7	1.84	1.77	3.67	
	Red-wattled lapwing	Charadriiformes	88	2.19	0.94	1.19	85.92	2.18	1.2	2.8	-	Abbasi et al. (2015a)
Multan	House crow	Passeriformes	0.23	108.2	22.86	0.15	0.71	229.3	12.47	0.83	4.08	This study
Southern Punjab	Same as above	Accipitriformes	4.06	59.82	8.06	0.86	12.42	-	-	-	7.63	Nighat et al. (2013)
	Same as above	Falconiformes	29.5	174.1	24.05	2.63	194.2	-	-	-	13.36	
Bahawalpur	Falco biarmicus jugger	Falconiformes		113.8	2.76	0.08	-	-	-	1.67	-	Movalli (2000)
Soon Valley	House crow	Passeriformes	0.0	114	3.37	0.13	0.3	211.9	9.7	0.37	9.03	This study
	Common pochard	Anseriformes	-	69.99	1.44	0.5	1.61	78.21	1.69	1.43	2.03	Abbasi et al. (2015b)
	Mallard	Anseriformes	-	94.59	1.85	1.16	1.85	122.6	1.5	1.69	2.99	
	Ruddy shelduck	Anseriformes	-	81.05	1.37	0.33	1.54	115.9	1.67	1.78	2.19	
	Gadwall	Anseriformes	-	83.43	1.34	0.99	0.51	123.6	1.56	1.72	2.466	
	Common kingfisher	Anseriformes	-	90.64	2.79	0.67	1.58	104.1	2.51	1.44	3.46	
	Common pochard, Common teal, Pintail	Anseriformes	-	74.14	2.32	0.95	1.8	102.2	2.3	1.67	3.11	Abbasi et al. (2015a)
	White wagtail, gray wagtail, citrine wagtail	Passeriformes	-	64.16	2.55	1.52	1.52	87.98	2.04	1.78	3.51	
	Common myna	Passeriformes	-	91.05	2.15	0.79	1.89	112	1.97	1.11	2.19	
Sargodha	House sparrow	Passeriformes	-	14.78	2.67	1.81	-	-	0.5	-	0.14	Mustafa et al. (2015)
	Black kite	Accipitriformes	-	18.76	2.4	0.13	-	-	0.63	-	0.13	
Gujar Khan	House crow	Passeriformes	0.0	159	11.55	0.2	1.35	124.3	13.75	1.5	10.15	This study
Northern Punjab	Same as above	Accipitriformes	6.19	768.1	16.3	0.82	33.58	-	-	-	12.76	Nighat et al. (2013)
	Same as above	Falconiformes	18.88	163.6	32.89	4.04	120.6	-	-	-	6.67	
	Same as above	Passeriformes	6.43	90.75	6.09	1.28	68.44	-	-	-	7.61	

Table 2 (continued)

Location	Species	Order	As	Zn	Pb	Cd	Ni	Fe	Mn	Cr	Cu	References
Karachi	House crow	Passeriformes	0.0	184.8	20.34	0.21	1.17	220.5	8.77	1.49	11.76	This study
	Falco biarmicus jugger	Falconiformes	-	104	ND	ND	-	-	-	2.31	-	Movalli (2000)
Jacobabad	House crow	Passeriformes	0.31	162.3	14.76	0.25	4.69	442.6	20.35	7.2	4.24	This study
	Falco biarmicus jugger	Falconiformes	-		1.28	0.11	-	-	-	-	-	Movalli (2000)

Fe and Zn were found to be the most abundant metals in the house crow feathers in common with other terrestrial birds from the studied areas (Table 2) and that reflects the ample bioavailability of these metals in the local environments (Abbasi et al., 2015a; Abdullah et al., 2015). Malik and Zeb (2009) suggested waste disposal from heavy industry as the root cause of environmental contamination in industrial areas, but the prevalence of Fe and Zn in the birds from rural and less industrialized areas of Punjab and northern mountainous ranges in the Khyber Pakhtunkhwa suggested input from natural sediments and this is upheld by geological studies (Khan et al., 2006; Muhammad et al., 2011; Nighat et al., 2013; Abbasi et al., 2015a; Abbasi et al., 2015b). Zn is an essential element of the body and necessary for different metabolic reactions, but deposition of excessive Zn in liver, kidney, and heart was diagnosed in birds and animals with clinical symptoms and histopathological injuries (Carpenter et al., 2004; Hussain & Tabassum, 2019; Kar et al., 2015). However, consistently high Zn and Fe concentrations were also measured in feathers among various crow species from other parts of the world without any reported incident of Zn toxicity (Table S1). Urban birds are usually more exposed to atmospheric anthropogenic pollutants compared to wild counterparts. Thus, elevated Fe and Zn levels in feathers may not solely represent long-term dietary uptake and accumulation but could also reflect contributions by the exogenous deposition from ambient atmospheric pollutants or from contaminated soil during foraging that is fixed by uropygial gland secretion (Dauwe et al., 2005; Jaspers et al., 2004).

Pb and Cd are non-essential elements and widely accepted as indicators of anthropogenic pollution (Scheifler, 2006). Pb was high in all urban birds and well above the concentrations (> 9 mg/kg) reported in urban European Blackbirds associated with impaired breeding success (Fritsch et al., 2019) while Cd was lower than the level (> 2 mg/kg) found in seabirds having lethal effects (Burger & Gochfeld, 2000b). At the studied sites, concentrations of Pb were considerably higher in house crows than the smaller terrestrial birds but were found to be comparable to the raptors (Table 2). A human biomonitoring study from Lahore also demonstrated a higher level of Pb in blood, nails and hairs than the other metals studied (Bibi et al., 2015). Similarly, the concentration of airborne Pb in particulate matter (PM_{10}) has been persistently reported to be high within several metropolitan areas of Pakistan (Von Schneidemesser et al., 2010; Rasheed, 2015; Mehmood et al., 2018; Ahmad et al., 2019).

Ni was found at higher levels in rural birds compared to urban companions, and this difference was significant in the north ($p < 0.023$) (Table 3). However, the observed Ni level in this study is lower than those reported in house crow feathers from other countries and also far less than the threshold set for birds (500 mg/kg) that can potentially disrupt their development and reproduction success (Outridge & Scheuhammer, 1993).

Mean concentrations of Mn, Cr, and Cu, industrial pollutants, were significantly elevated in the southern rural district of Jacobabad and the birds from northern industrial cities in comparison with the other two regions. This suggested the spread of the pollutants from the northern upstream industrial cities to the low-lying agricultural plains by the Indus irrigation canal system (Table 3). The interspecies comparison showed that house crows were substantially higher in Mn and Cu contamination than most of the terrestrial raptors (Table 2), but either equivalent to (for Mn) or less than piscivores aquatic birds studied in Punjab (Malik & Zeb, 2009; Ullah et al., 2014; Abdullah et al., 2015). Similarly different accumulation quantities were observed in different crow species and in different body tissues too (Table S1). It suggested that the accumulation of these metals is highly influenced by the quantity of metal exposure in different environments, different feeding habits, and disproportionate metal accumulation preference in different body tissues. At the same time, the Cr levels in house crow were the same as other species from our studied areas except in Jacobabad where the measured quantity (7.20 mg/kg) is the highest among terrestrial birds in Pakistan. A substantially higher concentration of Cu (139 mg/kg) and Cr (76.47 mg/kg) has been reported by Manjula et al. (2015) in house crows from industrial urban areas of Tiruchirappalli, India where several leather tanneries are situated (Table S1). The absence of any reported Cu toxicity incident in the species suggested that crows like several other species who regurgitate pellets frequently are not likely to be adversely affected by *Cu* ingestion (Franson et al., 2012).

Table 3 Pairwise comparison (Kruskal-Wallis *H* test) of average concentration of heavy metals among four bird groups

	Significance	Adjusted significance
Lead	$p = 0.000$	
North urban-north rural		0.000
North-rural-south urban		0.027
Nickel	$p = 0.002$	
North urban-north rural		0.023
Iron	$p = 0.000$	
South urban-north urban		0.000
South rural-north rural		0.000
Manganese	$p = 0.000$	
North urban-south urban		0.048
South rural-north rural		0.001
South rural-south urban		0.000
Chromium	$p = 0.003$	
South rural-north rural		0.031
South rural-south urban		0.019
Copper	$p = 0.000$	
North urban-south urban		0.000
North rural-south urban		0.002
South rural-south urban		0.000

Correlation among metals and source identification

Significant and positive correlations were highlighted among Zn, Cu, Mn and Ni, Cr within four bird groups at the 0.05 significance level by Spearman 2-tailed correlation analysis (Table S2). The only significant negative correlations were observed for As with Zn ($r = -0.402$, $p < 0.05$) and Cd ($r = -0.398$, $p < 0.05$) in the north urban region and with Ni ($r = -0.490$, $p < 0.05$) in the south rural bird group. The same metals have positive correlations in the north rural group. The reason for these opposite trends is unknown.

Four principal components (PCs) were extracted out by PCA that cumulatively explained 80.46% of the variance among four birds groups (Table S3). PC1 having eigenvalue 2.81 accounted for 31.28% of the variance with the positive loading of Zn, Pb, Cd and Mn metals. Excotoxicological studies from Pakistan and neighboring countries have reported the high accumulation of these metals in urban environments and related their anthropogenic input from industrial waste and traffic exhaust in cities (Qadir & Malik, 2009; Qadir et al., 2008; Yadav et al., 2019). PC2 with eigenvalue 2.08 accounted for 23.12% variance among bird groups and represented strong loading of Ni, Cr, and Cu. Ni and Cr are important constituents of traffic emission, while agricultural activities can influence the Cu level in areas having extensive cultivation (Johansson et al., 2009; Möller et al., 2005). Khan et al. (2013b), in his analysis of drinking water in the Swat valley location that we also sampled in this study, related the presence of these three metals to the erosion of mafic and ultramafic rocks and mining activities in that areas. Thus, Ni, Cr, and Cu can be bioavailable in the local environment through both natural and anthropogenic sources. A PCA bi-plot (Fig. 3) was drawn between the first two principal components. Birds from the south rural and north urban areas were more loaded along PC1 metals (Zn, Fe, Pb, Cd, Mn, and As) known to have anthropogenic and natural input from rock material, while PC2 metals (Ni, Cr, and Cu) were more differentiating metals in north rural birds. Results of hierarchical cluster analysis (HACA) well supported the PCA inferences. Three metals clusters were identified where Ni, Cr, and Cu were grouped together distinctly from the other two clusters of all other metals (Fig. 4). In general, the multivariate analysis identified major contribution of anthropogenic sources of pollutants in the three birds groups except north rural birds where the natural soil composition may also contribute to the bioavailability of heavy metals in the local environments that can be taken up in food chains.

Spatial distribution of heavy metals in rural and urban Pakistan

We found significant differential accumulation of Pb, Ni, Fe, Mn, Cr, and Cu among the four bird groups for the accumulation of in feathers (Table 3). Zn, Pb, Cd, Fe, and Mn were found consistently high in urban birds in both southern and northern regions and also in rural irrigated agricultural areas in low-lying Punjab and Sindh alluvial plains (south rural group). This highlights the role of the Indus irrigation system in the dissemination of urban industrial and municipal waste pollutants from industrialized cities located in upper Punjab (north) to far off agricultural plains. Numerous ecotoxicology studies have detected the levels of these heavy metals in the drinking water, soils, and vegetations in those areas that are high

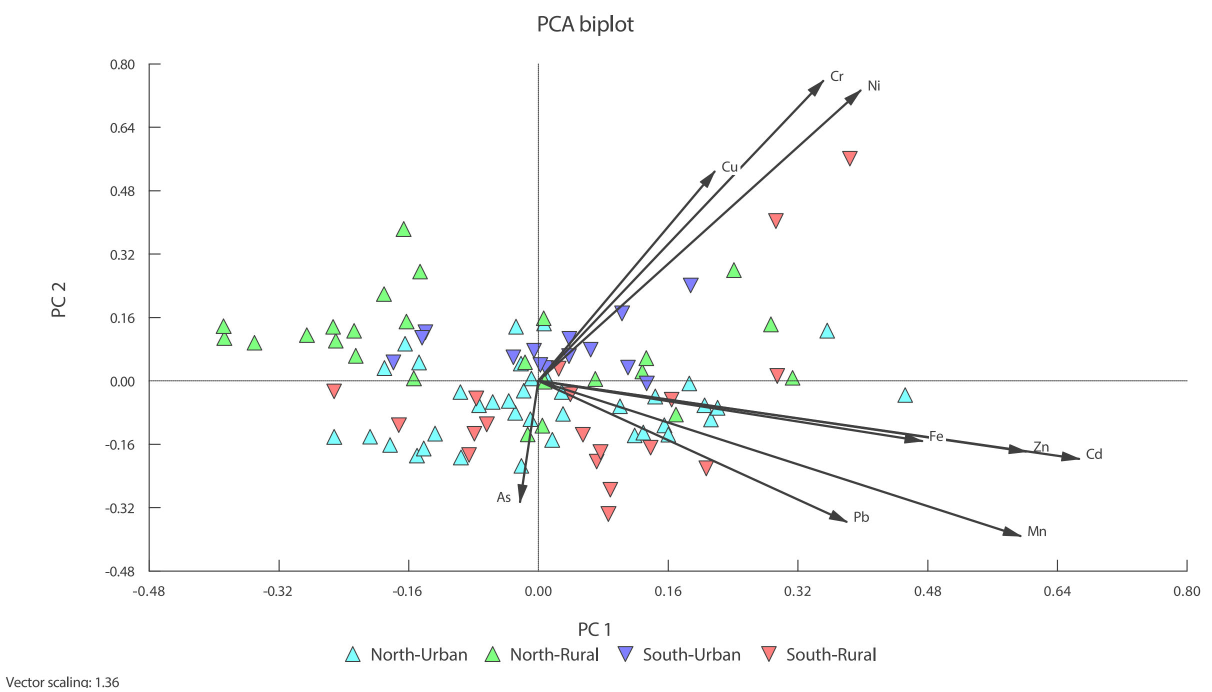

Fig. 3 PCA biplot inferring relationship of different metals and distribution in four habitats

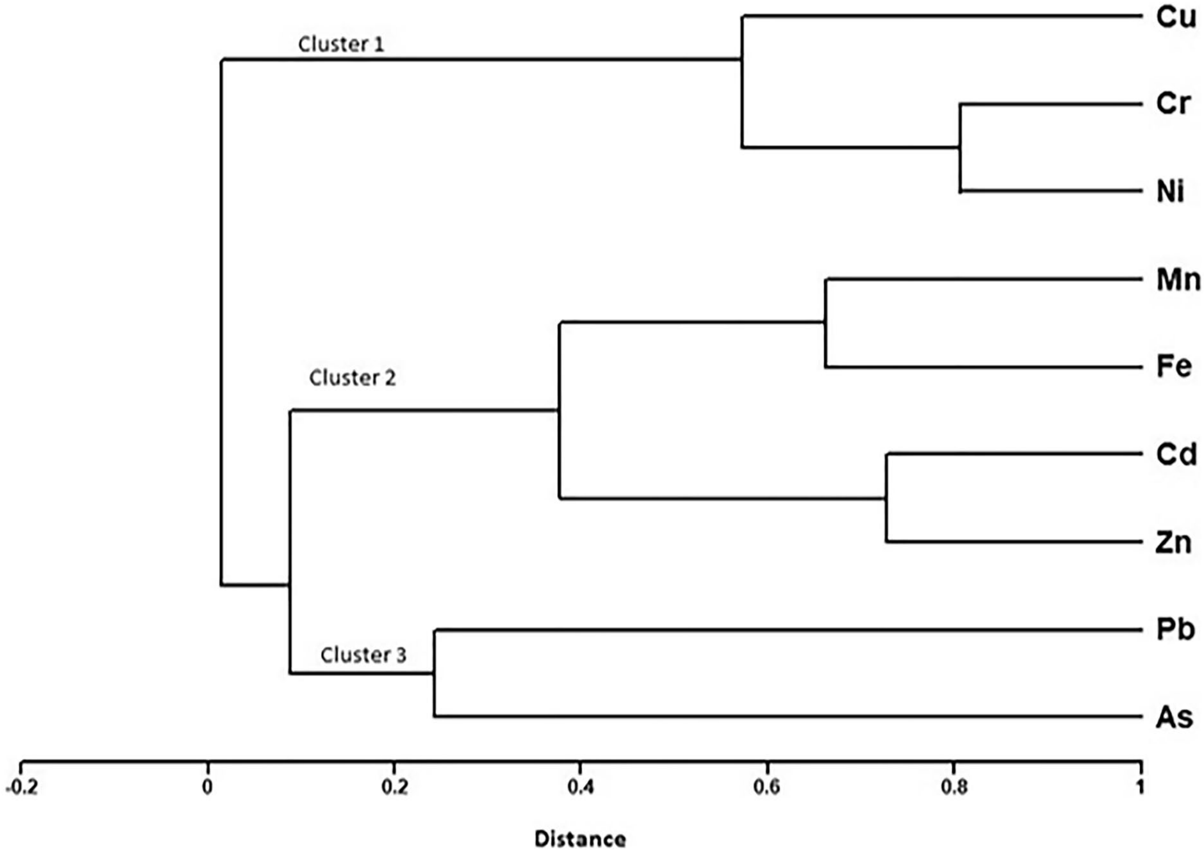

Fig. 4 Dendrogram of hierarchical clustering of metals obtained through UPGMA linkage method and Spearman coefficient distance matrix (distances show degree of association between metals and clusters)

above the prescribed safe limits (Farooqi et al., 2007; Batool et al., 2019; Jam, 2019). The north rural birds from the mountainous rural areas that are not the recipient of industrial waste from the central irrigation system accumulated less of the abovementioned metals but accrued Ni, Cr, and Cu more than the other three groups. Those may be available in the local environment there by the natural rock materials and mining as well as by agricultural activities such as extensive use of pesticides and fungicides on fruit orchards (Khan, 2013a; Khan et al., 2013b). Overall, Punjab and Sindh provinces were ranked higher than Khyber Pakhtunkhwa for metal contamination among house crows.

Biomonitoring potential of house crow

Avian biomonitoring studies conducted in Pakistan have been biased toward the raptors and wetland birds compared to the urban bird species (Table 2). Carnivores and piscivores harbored more toxins in their bodies because of their higher trophic levels in the food chains and longer life span that increase vulnerability to bioaccumulate contaminants (Finger et al., 2016; Szynkowska et al., 2018). Cattle egrets and pond herons were mostly studied from the wetlands of Punjab and Sindh. These invasive piscivorous species demonstrated substantial metal toxicity that was much higher than house crows (Boncompagni et al., 2003; Malik & Zeb, 2009; Abdullah et al., 2015). Abbasi et al. (2015b) analyzed some of those migratory species from the wetland in Soon Valley, a location that we also sampled in our study. The heavy metal burden in those migratory birds was less than in house crows and may be due to the seasonal exposure to the local anthropogenic pollutants and different dietary habits, as the studied *Anseriformes* are mostly herbivorous and some eat small fish, insects, and plankton.

Likewise in several species of raptor families (*Falconidae* and *Accipitridae*) employed for biomonitoring, metal contamination levels were several fold higher than in house crows from the same studied areas (Nighat et al., 2013). On the other hand, house crow, an easily sampled species, demonstrated significant differences in the metal accumulation levels in urban and rural areas when studied in an Indian district Tiruchirappalli (Manjula et al., 2015) and industrial port areas in Malaysia (Table S1) (Janaydeh et al., 2018).

Toxicity potential

Of all the metals, Pb concentration was above the threshold for toxic effects at all except two rural sites, while Cr was higher in the birds from the southern rural district (Table 4). The threshold levels used for comparison included the metal concentrations found in dead birds during controlled or field studies and the maximum tolerable levels (MTL) of the heavy metals determined by the National Research Council (2006) for poultry. Pb is a broad-spectrum metabolic poison, and > 2 mg/kg liver concentration was found responsible for inducing oxidative stress and liver toxicity in birds, while > 4 mg/kg feather concentration in seagulls was associated with thermoregulation impairment, low survival rate in nestlings, and delayed recognition of siblings (Pain et al., 1995; Gochfeld, 2000; Kanwal et al., 2020). Similarly, Cr toxicity can potentially induce teratogenic effects in birds such as twisted limbs, neck defects and hemorrhage, altered growth rates, and behavior changes (Malik & Zeb, 2009; Einoder et al., 2018). Thus, it is suggested that the fitness and survival of house crow and several other birds in the same habitats where the risk of contamination is high may be compromised.

Limitations of the study

Although feathers have been proved a nondestructive and relatively easy sampling means to measure pollutant loads in birds, there are some confounding factors that need to be considered in toxicological studies. Most prominently, the exogenous deposition of airborne particulate contaminants has been reported in feathers (Dauwe et al., 2002). Feathers are more prone to accrue metals such as Ni, Pb, Zn, and Al externally via deposition while foraging in contaminated soil or flying in polluted air (Dauwe et al., 2002). Although this exogenous deposition could well represent environmental pollution, it may skew the results related to internal bioaccumulation (Jaspers et al., 2004). We attempted to minimize this artifact by implementing a thorough pre-analysis wash of feathers with water and acetone as recommended by Burger and Gochfeld (2000b). Furthermore, the presence of relatively higher levels of some metals in feathers than the internal organs

Table 4 Comparing the heavy metal concentrations (mg/kg) in house crow to MLT values

Elements	Required nutrient	Concern for animal health	Our highest results (mg/kg)	MLT[a] (mg/kg)	Avian studies (mg/kg)	References
As	Possibly	Medium	0.31 (Jacobabad)	30	13.9 (feathers)	Sánchez-Virosta et al. (2018)
Zn	Yes	Medium	247.96 (Lahore)	500	524 (Liver) 453 (Kidney)	Taggart et al. (2009) Gasaway and Buss (1972)
Pb	No	High	22.86 (Multan)	10	9 (tail feathers)	Fritsch et al. (2019)
Cd	No	high	0.55 (Lahore)	10	>2 (feathers)	Burger and Gochfeld (2000a)
Ni	Possibly	Low	4.69 (Jacobabad)	250	>3 (liver) >10 (kidney)	Outridge and Scheuhammer (1993)
Fe	Yes	Medium	442.64 (Jacobabad)	500	NA	
Mn	Yes	Low	20.35 (Jacobabad)	2000	NA	
Cr	Yes	Low	7.20 (Jacobabad)	500	2.8 (feathers)	Burger and Gochfeld (2000a)
Cu	Yes	High	11.76 (Karachi)	250	NA	

suggests different routes of managing metals in the birds (Table S1). Certain metals like Pb, Cd, and Cr get chemically bonded to the keratin structure, and Pd and Zn seem to be incorporated into melanin-pigmented feathers more than non-melanin feathers (Janaydeh et al., 2018). It may also project the usage of feathers as an external tissue to store and detoxify metals in birds. Thus, it would be vital to consider these sources of variations in comparative or monitoring studies.

Conclusion

This is the first detailed study of trace metal contamination in an urban-dwelling bird species, across Pakistan under various levels of urbanization and pollution gradients. The birds from the industrialized cites and in rural agricultural areas that are irrigated through the central Indus Irrigation system were equal in metal contamination levels for Zn, Pb, Cd, Fe, and Mn. In mountainous rural areas, the influx of Ni, Cr, and Cu from the parent rock material and agricultural activities was detected through metal profiling of birds. House crow feathers were found accumulate more Zn and Fe than other bird species. Pb was highlighted at several places with concentrations well exceeding the toxicity threshold. The house crow is ranked in the middle on metal accumulation between large carnivores or piscivores and small insectivores or granivores, and this may be due to its being an omnivore. In addition, the house crow, being a resident species, is suggested to be a better bioindicator choice for site-specific differences than carnivores that bioaccumulate more metals due to higher position in the food chain. For migratory birds, a limitation for biomonitoring is that it is rarely possible to define the feeding areas from which they accumulated the metals. The results of our study are of significance as they provide baseline information about heavy metal accumulation in urban birds and can also be used as comparative data for wild passerines that remained underrepresented in avian toxicological studies. House crows are widespread throughout South Asia, South East Asia, the Arabian Peninsula, and East Africa and may facilitate the comparison among different regions and for long-term monitoring of environmental hazards. These sedentary birds live in close proximity to human settlements, and these results can be extended to identify potential health risks of heavy metal pollution in human populations in their vicinity.

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Declarations

Ethics approval All applicable international, national, and institutional guidelines were followed for dispatching samples from Pakistan and while handling in the laboratory for analysis.

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