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Trace Element Concentrations in Feathers of Feral Pigeons (*Columba livia*) and Hooded Crows (*Corvus cornix*): Interspecific Patterns and Non-Invasive Biomonitoring across Urban Iran

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ABSTRACT

Aims: This study evaluated feather element concentrations in two synanthropic avian species, the Rock Dove (*Columba livia*) and the Hooded Crow (*Corvus cornix*), across three Iranian cities with different urbanization levels (Tehran, Mashhad, and Sabzevar).
Materials & Methods: Tail feathers were collected from *C. livia* and *C. cornix* in Tehran, Mashhad, and Sabzevar from road casualties, birds confiscated from illegal hunting, and specimens provided by local conservationists between 2020 and 2022. The outermost tail feather from each specimen was analyzed for Lead (Pb), Copper (Cu), and Zinc (Zn). Metal concentrations were compared among cities, between species, and between age classes in *C. livia*.
Findings: Mean concentrations in *C. livia* were 0.21, 8.89, and 10.18 $\mu g.g^{-1}$ dry weight (dw) for Pb, Cu, and Zn, respectively, whereas those in *C. cornix* were 0.55, 9.33, and 10.41 $\mu g.g^{-1}$ dw. Statistical analyses revealed significant inter-city variation in both species. Feather trace element concentrations differed significantly between species, but no age-dependent variation (juveniles vs. adults) was observed in *C. livia*. All measured concentrations were below the cited avian feather reference values for Pb (4.0 $\mu g.g^{-1}$), Cu (250 $\mu g.g^{-1}$), and Zn (500 $\mu g.g^{-1}$).
Conclusion: Feather trace element concentrations indicated low baseline metal levels across the studied cities. To our knowledge, this study provides the first baseline data on feather trace element concentrations in *C. cornix* from urban areas in Iran.

Keywords: Biomonitoring; Urban Contamination; Pollutants; Toxic Elements; Urban Ecosystem.

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Introduction

The rapid expansion of urban centers has intensified anthropogenic pollutant release, creating complex toxicological challenges in urban ecosystems. This trend, driven by population growth and human activities such as industrial production, agriculture, and transportation, has increased the diversity and accumulation of environmental contaminants. Trace metals are of particular concern because of their environmental persistence and potential adverse effects on human and wildlife health. Although essential metals, such as Zinc (Zn) and Copper (Cu), are required for normal physiological functions, excessive exposure can cause oxidative stress and cytotoxicity. In contrast, non-essential metals such as Lead (Pb) are highly toxic even at low exposure levels, requiring rigorous risk assessment and systematic environmental monitoring [1,2].

Trace metals originate from multiple urban sources, including vehicle emissions, the resuspension of settled road dust, industrial effluents, and municipal waste [3,4]. Chronic exposure through inhalation, ingestion, or dermal contact can cause adverse effects, including impaired reproductive success, neurological deficits, metabolic disruption, and immunotoxicity [5–9]. Given these risks, avian bioindicators have gained prominence in urban ecotoxicology. Birds are effective models because of their wide distribution, diverse foraging behaviors, and occurrence at different trophic levels. Previous studies have demonstrated the value of avian species as biomonitors worldwide, including the black swan (*Cygnus atratus*) in Australia [10], the European starling (*Sturnus vulgaris*) in the United States [11], and the house crow (*Corvus splendens*) in Pakistan.[12] the house crow (Corvus splendens).

Among biological matrices, feathers are particularly useful for biomonitoring because they can be collected with minimal disturbance, are easy to transport and store, and may provide an integrated record of metal exposure during feather growth. [8,13,14] The intensive development of industry and human population results in large amounts of different xenobiotic emitted into individual ecosystem components. As a consequence, monitoring of the level of pollution of particular elements of the environment by exotoxins has become a common interest. The determination of environmental changes by different types of biological indicators is called bioindication, which is used as one of the basic methods in the monitoring of environmental pollution. The following review paper contains comprehensive information about the use of bird feathers to assess the environmental contamination level. Types of contaminants (trace metals, microplastics, persistent organic pollutants. However, interpreting metal concentrations in feathers requires careful consideration of variables such as feather type, molt/growth status, and sampling timing. Furthermore, feather concentrations reflect both endogenous incorporation during growth and potential exogenous deposition; thus, while standardized washing procedures were implemented to minimize superficial contamination, they cannot eliminate this source of bias. To assess the bioavailability of urban contaminants, we selected two synanthropic species with distinct ecological niches: the feral pigeon (*Columba livia*), a ground-foraging granivore, and the hooded crow (*Corvus cornix*), an opportunistic omnivore with a higher trophic position

[12–14]the house crow (Corvus splendens. This pairwise comparison was designed to contrast exposure pathways linked to (i) ground-level foraging and frequent contact with urban dust/soil and road-deposited particulates in pigeons versus (ii) broader dietary breadth in crows, including scavenging and consumption of human food waste and animal-derived items, which may increase the likelihood of ingesting metals via multiple trophic routes. Accordingly, interspecific differences in feather Pb, Cu, and Zn were interpreted not merely as "species effects," but as ecologically meaningful signals of how diet composition and foraging microhabitats shape exposure in urban landscapes. Finally, these species were sampled across Tehran, Mashhad, and Sabzevar, cities selected to represent a clear gradient of urbanization based on documented differences in human population density, industrial activities, and vehicular traffic volume [15–18].

The objectives of this study were to quantify Pb, Cu, and Zn concentrations in feathers, assess potential age-related differences between juvenile and adult birds, and interpret the results in relation to established ecotoxicological thresholds, while acknowledging the limitations of these thresholds regarding matrix and species specificity. It was hypothesized that birds sampled along an anthropogenic contamination gradient characterized by documented variations in vehicular traffic and industrial activity would exhibit distinct metal concentrations, after accounting for potential confounding factors such as species-specific diet and habitat use. Given the paucity of comparative data on urban avian contaminants in Iran, this study addresses critical knowledge gaps regarding interspecific variation and spatial pollution patterns, providing essential baseline data for *C. cornix* and broadening the scope of urban avian ecotoxicology.

Materials & Methods

Study Area and Sample Collection

The study was conducted across three Iranian cities exhibiting distinct gradients of anthropogenic activity and urbanization: Tehran (the national capital and an industrialized megacity characterized by dense vehicular traffic), Mashhad (a major regional commercial metropolis), and Sabzevar (a smaller urban center with lower industrial density) (Figure 1). Avian specimens were opportunistically collected over two years (mid-2020 to mid-2022) in accordance with established non-invasive ecotoxicological frameworks that utilize salvaged carcasses and road casualties to avoid intentional wildlife harvesting [19,20]. Carcasses were obtained from three documented sources of mortality: vehicular collisions, specimens lawfully confiscated from illegal hunting by regional Department of Environment (DOE) authorities, and salvaged specimens provided by local wildlife conservationists. Only fresh carcasses with intact plumage and no visible signs of decomposition or severe feather degradation were sampled to ensure analytical integrity. Because specimens were retrieved opportunistically across broader municipal perimeters without high-resolution GPS coordinates, spatial analyses reflect regional urban baselines rather than fine-scale micro-spatial contamination gradients. Furthermore, pooling samples across the sampling period integrated baseline exposure, but fine-scale seasonal and interannual fluctuations could not

Table 1) Summary of collected feather samples of *Columba livia* and *Corvus cornix* across the three study cities.

Species	City	Total Samples (n)	Adults (n)	Juveniles (n)	Road Casualties / Found Dead (%)
C. livia	Tehran	10	5	5	20%
	Mashhad	10	5	5	10%
	Sabzevar	12	5	7	33%
C. cornix	Tehran	8	Undetermined*	Undetermined*	0%
	Mashhad	9	Undetermined*	Undetermined*	33%
	Sabzevar	9	Undetermined*	Undetermined*	33%

Note: *Age and sex of *C. cornix* were not morphologically distinguishable.

be resolved, an intrinsic constraint of opportunistic sampling.

In total, 58 individuals comprising 32 feral pigeons (*Columba livia*; primarily granivorous) and 26 hooded crows (*Corvus cornix*; opportunistic omnivore) were examined to evaluate species-specific exposure pathways. To minimize intra-individual variation associated with molt chronology, the outermost right tail feather was systematically selected from each specimen, thereby yielding a standardized matrix with consistent growth history for elemental determination. Collected feathers were placed in pre-cleaned paper envelopes and stored at ambient temperature until preparation. Details of sample distribution, sources of mortality, and age classes—determined in *C. livia* from standardized morphological markers (e.g., cere development and iridescence of neck feathers) are provided in Table 1. In contrast, owing to the absence of external sexual dimorphism and the lack of reliable post-mortem diagnostic plumage criteria in salvaged carcasses, neither sex nor age class could be determined for *C. cornix*, representing an unquantified source of biological variation.



Figure 1) Location of the provinces and cities included in this study.

Sample Preparation and Chemical Analysis

To remove loosely adhered exogenous dust and atmospheric particulates, the sampled outermost rectrices were cleaned following validated avian ecotoxicological protocols [20,21]. Feathers underwent three alternating washing cycles with deionized water and analytical-grade acetone in an ultrasonic bath to remove surface contaminants without extracting endogenous intracellular trace elements, followed by a final thorough rinse with deionized water. Although this protocol effectively eliminates macroscopic debris and external dust, chemically bound atmospheric deposition may partially

resist non-destructive chemical washing, which remains an acknowledged property of feather biomonitoring matrices.

Cleaned feathers were dried in an oven at 60 °C for 24 h to constant weight and homogenized into fine fragments using trace-metal-free stainless-steel scissors. Approximately 1 g of homogenized feather material was weighed (±0.001 g) for wet acid digestion and pre-digested with 8 mL of 65% HNO_3 (Suprapur, Merck) for 12 h at room temperature, followed by the addition of 2 mL of 60% $HClO_4$. Digestion was performed in a water bath using a programmed temperature profile (40 °C for 1 h, followed by 140 °C for 3 h) to ensure complete decomposition of the keratinous matrix [20,21]. The resulting digests were filtered through acid-leached Whatman No. 42 filter paper and brought to a final volume of 10 mL with deionized water. Concentrations of Lead (Pb), Copper (Cu), and Zinc (Zn) were then determined by graphite furnace atomic absorption spectrometry (GFAAS; Shimadzu AA-6800) at the Central Analytical Laboratory of Jovain Agricultural and Industrial Co., in accordance with established standard operating procedures.

Quality Assurance and Quality Control (QA/QC)

To minimize potential contamination, all glassware, PTFE digestion vessels, and polyethylene containers were acid-washed by immersion in 10% HNO_3 for 24 h and rinsed thoroughly with ultrapure deionized water before use. Instrument drift and calibration stability were monitored periodically throughout the analytical sequence; linear calibration curves for all target elements displayed high goodness-of-fit ($R^2 > 0.992$). Procedural reagent blanks (n = 3 per digestion batch) were incorporated into every analytical batch to assess reagent-borne baseline interference, and all final elemental concentrations were blank-corrected. Analytical accuracy was assessed using the certified reference material NCS DC73347a (Trace Elements in Human Hair, China National Analysis Center; certified values: Pb: 5.7 ± 0.5 $\mu g.g^{-1}$, Cu: 14.3 ± 1.6 $\mu g.g^{-1}$, and Zn: 137 ± 9 $\mu g.g^{-1}$), processed strictly through the same acid digestion and analytical protocol. Recovery rates for the CRM were within acceptable analytical ranges: 91%–98% for Pb, 94%–104% for Cu, and 95%–107% for Zn. Analytical precision was evaluated through triplicate analyses of randomly selected feather samples; relative standard deviations (RSDs) were consistently below 7%. The limits of detection (LOD) and quantification (LOQ) were empirically established on a solid dry-weight basis as 3 and 10 times the standard deviation (and) of eleven independent procedural blanks, accounting for sample mass and the final 10 mL dilution volume. The resulting method LOQs were 0.020 $\mu g.g^{-1}$ dw for Pb, 0.105 $\mu g.g^{-1}$ dw for Cu, and 2.731 $\mu g.g^{-1}$ dw for Zn. Importantly, all measured feather elemental concentrations across both species and all sampling locations exceeded their respective LOQs; consequently, no left-censored substitution procedures (e.g., replacement by) were required in statistical evaluations. Elemental concentrations are expressed on a dry-weight basis as $\mu g.g^{-1}$ dw.

Statistical Analysis

The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Differences in metal concentrations among the three cities were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's post hoc test. Differences between *C. livia* and *C. cornix*, as well as age-related differences in *C. livia*, were assessed using independent-samples *t*-tests.

All statistical analyses were performed at a significance threshold of $p < 0.05$.

Findings

Spatial Patterns and Interspecific Variations in Feather Trace Element Concentration

Feather trace element concentrations (Pb, Cu, and Zn) in feral pigeons (*Columba livia*) and hooded crows (*Corvus cornix*) demonstrated pronounced spatial variations across the three surveyed metropolitan areas (Sabzevar, Mashhad, and Tehran; Figure 2 and Figure 3). Overall, all quantified metal concentrations fell consistently above the analytical limits of quantification (LOQ). In *C. livia*, the highest mean Pb concentration was recorded in Tehran (0.45 ± 0.18 $\mu g.g^{-1}$ dw; all data are presented as mean ± standard error, SE), while Cu reached peak concentrations in Mashhad (9.48 ± 0.15 $\mu g.g^{-1}$ dw) and Tehran (9.45 ± 0.29 $\mu g.g^{-1}$ dw), and Zn displayed its maximum value in Mashhad (11.14 ± 0.32 $\mu g.g^{-1}$ dw**;** Table S1**).** For *C. cornix*, the highest mean concentrations for all three quantified elements were consistently documented in Tehran (Pb: 1.23 ± 0.07 $\mu g.g^{-1}$ dw; Cu: 10.29 ± 0.14 $\mu g.g^{-1}$ dw; Zn: 11.42 ± 0.16 $\mu g.g^{-1}$ dw**;** Figure 2 and Table S1**).** Statistical analyses revealed significant inter-city differences in feather metal concentrations for both species (Table 2). Specifically, in *C. livia*, significant spatial variations were found for Pb (p=0.023), Cu (p=0.034), and Zn (p<0.001) (Figure 3). In *C. cornix*, marked geographical disparities were observed for Pb (p<0.001), Cu (p=0.031), and Zn (p=0.030).

Interspecific comparisons of Pb, Cu, and Zn concentrations are presented in Table S1. Significant differences ($p < 0.05$) in feather trace element concentrations between *C. livia* and *C. cornix* were detected in Tehran and Mashhad for all three analyzed elements. In Tehran and Mashhad, feather Pb concentrations were significantly higher in *C. cornix* than in *C. livia* ($p < 0.05$). Furthermore, *C. cornix* exhibited significantly higher Cu and Zn concentrations in Tehran, whereas in Mashhad, *C. livia* displayed significantly higher Cu and Zn levels. In contrast, no statistically significant interspecific differences were observed for any of the three elements in Sabzevar ($p < 0.05$; Table S1).

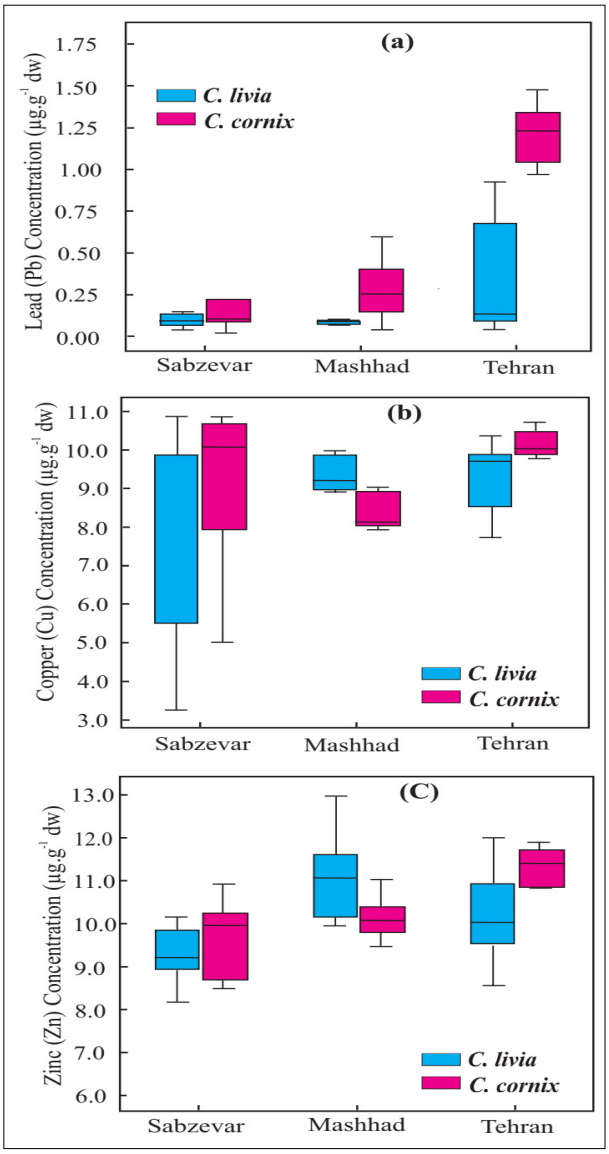


Figure 2) The concentrations of trace elements in tail feathers of *C. livia* and *C. cornix* across the three study areas (Sabzevar, Mashhad, and Tehran): (a) Lead (Pb), (b) Copper (Cu), and (c) Zinc (Zn). Values represent the mean standard error (SE).

Table 2) Differences in element concentrations in feather samples of *C. livia* and *C. cornix* from Sabzevar, Mashhad, and Tehran.

Species	Element	Parameter					
			Sum of Squares	df	Mean Square	F	Sig.
C. livia	Pb	Between Groups	0.89	2	0.45	4.30	0.023
		Within Groups	3.03	29	0.10		
		Total	3.93	31			
	Cu	Between Groups	22.54	2	11.27	3.82	0.034
		Within groups	85.65	29	2.95		
		Total	108.16	31			
	Zn	Between Groups	21.58	2	10.79	10.25	0.000
		Within Groups	30.54	29	1.05		
		Total	52.12	31			
C. cornix	Pb	Between Groups	5.45	2	2.73	76.80	0.000
		Within Groups	0.82	23	0.04		
		Total	6.27	25			
	Cu	Between Groups	13.97	2	6.99	4.06	0.031
		Within Groups	39.57	23	1.72		
		Total	53.54	25			
	Zn	Between Groups	14.11	2	7.06	4.12	0.030
		Within Groups	39.42	23	1.71		
		Total	53.53	25			

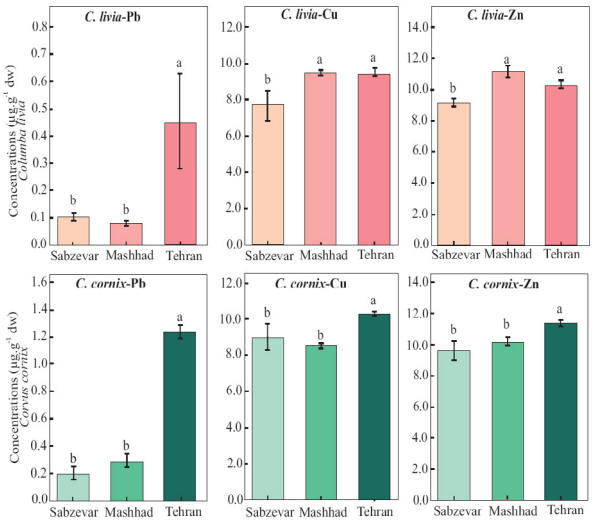


Figure 3) Mean concentrations (μg.g^{-1} dw) of Pb, Cu, and Zn in feather samples of *Columba livia* and *Corvus cornix* across the three study sites (Sabzevar, Mashhad, and Tehran). Error bars represent the standard error (SE). Different lowercase letters (a, b) above the bars indicate statistically significant differences ($p < 0.05$) among cities for each species.

Demographic Patterns and Comparison with Ecotoxicological Baselines

For adult and juvenile *C. livia*, mean ± SD feather concentrations were 0.25 ± 0.41 and 0.21 ± 0. 24 μg.g^{-1} dw for Pb; 9.20 ± 1.72 and 8.81 ± 1.86 μg.g^{-1} dw for Cu; and 10.18 ± 1.29 and 10.11 ± 1.29 μg.g^{-1} dw for Zn, respectively. No significant age-related differences were detected for any of the measured elements in *C. livia* (Figure 4). For *C. cornix*, sex and age classes could not be determined reliably from the salvaged carcasses (Table 1); hence, demographic comparisons were conducted exclusively for *C. livia*.

The measured feather trace element concentrations were compared with established ecotoxicological thresholds and dietary tolerance limits. Pb concentrations

across all sampled groups were consistently well below the avian toxicity threshold of 4.0 μg.g^{-1} dw. Similarly, overall Cu and Zn concentrations in all examined samples were markedly lower than the physiological limits established for avian species (250 μg.g^{-1} for Cu and 500 μg.g^{-1} for Zn).

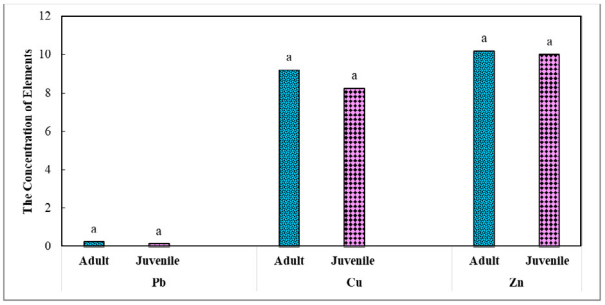


Figure 4) Comparison of Pb, Cu, and Zn concentrations (μg.g^{-1} dw) between adult and juvenile *C. livia* feather samples.

Discussion

Feather Pb, Cu, and Zn concentrations in *Columba livia* and *Corvus cornix* exhibited notable spatial variations across the three surveyed metropolitan areas (Tehran, Mashhad, and Sabzevar). As hypothesized, specimens from Sabzevar a smaller urban center with fewer industrial hubs and lower traffic density generally exhibited lower trace element concentrations than those from the two megacities. In *C. livia*, mean Cu and Zn concentrations were significantly lower in Sabzevar than in Tehran and Mashhad, whereas Pb peaked in Tehran. In *C. cornix*, Tehran specimens showed significantly higher concentrations of all three elements than those from Mashhad and Sabzevar. Importantly, because carcasses were retrieved opportunistically without exact coordinates or intra-urban spatial stratification, these geographic disparities reflect broad, macro-scale metropolitan exposure baselines rather than high-resolution intra-city or micro-spatial contamination gradients. Furthermore, environmental covariates (e.g., traffic volume, industrial emissions, and land-use metrics) were not quantified directly; hence, these geographic patterns represent integrated regional differences rather than definitive causal links to specific localized point sources.

The mean feather Pb concentrations in Tehran (0.45 ± 0.18 μg.g^{-1} dw in *C. livia* and 1.23 ± 0.07 μg.g^{-1} dw *in C. cornix*) were higher than those in Mashhad and Sabzevar, consistent with greater Pb exposure in the capital city. However, the study design does not identify the specific sources responsible for this variation [22,23]. These values remain lower than some levels reported in historical studies of large metropolitan areas; however, direct comparisons are constrained by differences in species, sampling periods, and analytical methodologies. While the national phase-out of leaded gasoline since the early 2000s and fleet modernizations provide a general historical context for modern baseline exposures, they cannot fully account for the observed variations without direct historical tracking. Because carcasses were retrieved opportunistically without precise spatial coordinates, the Tehran samples reflect a broad metropolitan baseline and may not capture high-resolution exposure gradients from congested street canyons. Furthermore, the lack of significant Pb differences between Mashhad and Sabzevar indicates that city size alone does not dictate avian exposure; local point sources, microclimate, and urban morphology likely exert overriding controls [22], aligning with prior context-dependent findings [3,23–26].

A central finding of this investigation is that feather concentrations of trace elements in both species exhibited distinct elemental

patterns when benchmarked against global biomonitoring literature (Table 3). Feather Cu concentrations recorded in the present work align closely with baseline ranges reported for urban feral pigeons in Paris [23] and Jaipur [27]. In sharp contrast, feather burdens of Pb and Zn in both *C. livia* and *C. cornix* remained markedly lower than those documented in heavily contaminated industrial habitats (Table 3). Most notably, in the industrial complex of Zanjan, Iran, feather levels of Pb (16.14 $\mu g.g^{-1}$ dw) and Zn (326.66 $\mu g.g^{-1}$ dw) exceeded our findings by up to an order of magnitude for Pb and thirty-fold for Zn [14], directly reflecting intensive atmospheric emissions from primary Lead–Zinc smelting facilities. Similarly, published Pb and Zn burdens in corvids from European and South Asian urban mosaics were substantially higher than those observed across Tehran, Mashhad, and Sabzevar [12,25,28]the house crow (Corvus splendens. Several methodological, physiological, and exposure-related factors readily account for these observed concentrations. First, the rigorous pre-analytical decontamination procedure employed here comprising sequential ultrasonic baths in acetone and high-purity deionized water effectively eliminated superficially adhered atmospheric particulates and road dust, thereby minimizing exogenous surface-borne bias [29].

In contrast, studies that employ superficial or water-only rinsing often report artificially inflated elemental burdens due to recalcitrant external particle deposition. In this context, although feather Zn concentrations in severely degraded environments frequently exceed 100 $\mu g.g^{-1}$ dw, baseline endogenous levels range from 7.0 to 25.0 $\mu g.g^{-1}$ $dw.g^{-1}$ dw, baseline endogenous levels ranging between 7.0 and 25.0 $\mu g.g^{-1}$ dw are widely recognized across diverse avian taxa following exhaustive washing protocols that isolate the internal keratin matrix. Second, for essential micronutrients such as Zn and Cu, systemic bioavailability is tightly buffered by homeostatic mechanisms, including metallothionein synthesis and hepatobiliary excretion [30,31]. These active regulatory pathways maintain circulating pools within strict physiological bounds under moderate environmental exposure, thereby constraining excessive elemental incorporation into growing feathers. Third, endogenous deposition into the feather shaft is strictly limited to the discrete developmental window of active blood supply; once keratinization is complete and follicle vascularization atrophies, the feather becomes metabolically quiescent and refractory to further internal transfer [32,33]. Finally, unlike studies concentrated around acute industrial point sources, our specimens were recovered from heterogeneous urban landscapes where wide home ranges and opportunistic foraging strategies likely dilute localized environmental exposure. From an ecotoxicological perspective, these baseline-like burdens indicate that feral pigeons and hooded crows in the surveyed Iranian cities are currently subject to sub-threshold trace element exposure, remaining well below established limits for overt clinical toxicity or reproductive impairment.

Interspecific variations reflect distinct niches and diets, though unquantified biological covariates must also be considered. In Tehran and Mashhad, *C. cornix* exhibited higher Pb burdens than *C. livia*, aligning with the crow's broader opportunistic omnivory versus the localized, granivorous habits of pigeons. However, because age and sex could

Table 3) Mean concentrations of Lead, Zinc, and Copper (μg.g^{-1}) reported in feathers of feral pigeons and crows across different international and regional biomonitoring studies.

Species		Element			Location	Reference
Common Name	Scientific Name	Pb	Cu	Zn		
Feral Pigeons[a]	*C. livia*	0.10	7.70	9.20	Sabzevar	This study
Feral Pigeons[a]	*C. livia*	0.10	9.50	11.10	Mashhad	This study
Feral Pigeons[a]	*C. livia*	0.50	9.50	10.30	Tehran	This study
Feral Pigeons[b]	*C. livia*	16.14	NA**	326.66	Industrial town, Zanjan, Iran	[14]
Feral Pigeons[b]	*C. livia*	1.76	NA	49.06	Control area, Zanjan, Iran	[14]
Feral Pigeons[a]	*C. livia*	7.70	10.40	NA	Hormod protected area, Iran	[34]
Feral Pigeons[a]	*C. livia*	3.22	NA	NA	Goiânia and Jataí, Brazil	[35]
Feral Pigeons[a]	*C. livia*	13.82	13.15	204.80	Paris, France	[23]
Feral Pigeons[a]	*C. livia*	2.37-3.0	7.05-9.07	27.14-35.13	Jaipur, India	[27]
Hooded Crow[a]	*C. cornix*	0.20	9.20	9.60	Sabzevar	This study
Hooded Crow[a]	*C. cornix*	0.30	8.50	10.20	Mashhad	This study
Hooded Crow[a]	*C. cornix*	1.20	10.30	11.40	Tehran	This study
Crows:[b] * **Hooded Crows, Carrion Crow and Rook**	*C. cornix, C. corone, and C. frugilegus*	4.33	26.96	118.58	Hungary	[28]
House Crow [a]	*C. splendens*	32.02	15.58	206.32	Selangor, Malaysia	[36]
House Crow [a]	*C. splendens*	13.95	6.82	75.04	Agricultural fields of Ludhiana, India	[25]
House Crow [a]	*C. splendens*	44.41	19.47	416.34	Agricultural fields of Sangrur district, India	[25]
House Crow [a]	*C. splendens*	19.11	6.61	248.00	Lahore, Pakistan	[12]
House Crow [a]	*C. splendens*	12.90	6.64	239.50	Okara, Pakistan	[12]
House Crow [a]	*C. splendens*	0.13	9.03	114.00	Soon Valley, Pakistan	[12]

Note: * Values for Hungarian crows represent pooled data for three species without species breakdown and **NA: Not Available. [a] Concentrations are reported on a dry weight basis (μg.g^{-1} dw), and [b] Units are reported simply as μg.g^{-1}.

not be determined for *C. cornix*, demographic asymmetry is a plausible confounder; the elevated elemental burdens in crows may partly stem from an unquantified proportion of older individuals with prolonged bioaccumulation histories across sequential molting cycles**.** For Cu and Zn, interspecific patterns were city-dependent (*C. cornix* dominated in Tehran, *C. livia* in Mashhad), reflecting local variations in food quality and waste management. Crucially, these disparities vanished in Sabzevar, indicating that lower anthropogenic pressure minimizes feeding-guild differentials.

No significant age-related differences were detected between adult and juvenile *C. livia*. While adult birds typically show higher heavy metal burdens in internal storage organs (e.g., liver or kidney), our findings align with feather biomonitoring literature reporting negligible or inconsistent age effects [10,28,37]. Because endogenous trace element incorporation is restricted to the discrete developmental window of active follicle vascularization, mature feathers reflect circulating blood pools strictly during synthesis rather than a cumulative lifetime body burden [29,33]. Once keratinization is complete, the feather shaft becomes metabolically quiescent. Furthermore, dietary overlap and shared foraging territories between fledged juveniles and adults within the same urban matrix likely minimize cohort-specific exposure disparities.

In contrast, age and sex could not be reliably determined in *C. cornix* due to sexual monomorphism and the variable condition of salvaged carcasses. We explicitly acknowledge this lack of age- and sex-disaggregated data as a biological limitation. Given that sex-specific hormonal kinetics and age-mediated exposure duration can influence metal uptake [10,38], the inability to stratify the corvid cohort introduces unaccounted biological variability that warrants cautious interpretation of our interspecific comparisons.

When interpreting these findings from an ecotoxicological perspective, comparisons with published threshold values provide useful preliminary context, although such benchmarks must be interpreted with considerable caution. For Lead, feather concentrations exceeding approximately 4.0 $\mu g.g^{-1}$ dw have historically been associated with adverse neurobehavioral, physiological, and reproductive impairments in wild birds [39,40]. While feather Pb levels in our sampled populations remained well below this indicative benchmark in all groups, this threshold originated primarily from specific bird species (e.g., larids and raptors) and is not uniformly applicable across all avian taxa, age classes, or ecological contexts. Similarly, while measured Cu and Zn concentrations were well below widely cited general tolerance limits (e.g., 250 $\mu g.g^{-1}$ dw for Cu and 500 $\mu g.g^{-1}$ dw for Zn; [12]the house crow (Corvus splendens), these guidelines largely reflect dietary or soft-tissue tolerances derived from captive or domestic models rather than validated feather-specific toxicological thresholds. Crucially, elemental concentrations falling below these generalized reference levels do not inherently demonstrate the absence of toxicological risk. Feathers capture circulating trace elements only during the restricted window of keratin synthesis and molt, rather than directly reflecting internal organ burdens (such as hepatic or renal stores). Furthermore, because this study did not assess specific health endpoints (e.g., body

condition, reproductive success) or internal physiological biomarkers (such as oxidative stress, ALAD inhibition, or metallothionein levels), feather concentrations alone cannot confirm the complete lack of adverse effects. Consequently, while the measured levels suggest comparatively low circulating exposure during the molt period, potential sub-clinical effects or chronic physiological stress cannot be excluded without direct physiological and tissue-level investigations. Future studies should include rural or peri-urban reference populations to distinguish urban inputs from regional geochemical background levels. Broader contamination gradients, additional contaminants (e.g., Cd, Ni, and organic pollutants), and biochemical biomarkers would further improve exposure assessment in synanthropic birds.

Limitations of the Study

Several methodological and biological limitations should be considered when interpreting these findings:

1. Residual Exogenous Contamination: Although feathers underwent rigorous ultrasonic washing in acetone and ultrapure water, microscopic particulates firmly embedded in the keratin matrix may resist non-destructive cleaning, meaning measured burdens could integrate minor external inputs alongside endogenous fractions.

2. Opportunistic Sampling and Spatial Resolution: Specimens were collected opportunistically from salvage sources without sub-meter GPS logging. Consequently, data delineate macro-spatial metropolitan baselines and cannot be interpreted as fine-scale micro-spatial contamination gradients.

3. Unquantified Demographic Covariates in *C. cornix*: While *C. livia* cohorts were stratified by age, the absence of sexual dimorphism and post-mortem carcass damage precluded age and sex determination in *C. cornix*, leaving potential age- or sex-dependent accumulation dynamics uncharacterized in this species.

4. Sample Size Constraints: Modest sample sizes in certain city-species cohorts may have limited the statistical power needed to detect subtle inter-cohort variation.

5. Contaminant Panel and Biological Endpoints: Analysis was restricted to Pb, Cu, and Zn without concurrent assessment of internal organ residues, histological lesions, or physiological biomarkers (e.g., ALAD activity, oxidative stress), precluding definitive conclusions regarding clinical or population-level toxicological risks.

Conclusion

This study demonstrated variations in feather concentrations of Lead (Pb), Copper (Cu), and Zinc (Zn) in *Columba livia* and *Corvus cornix* across different Iranian urban environments. These observed spatial patterns align with anticipated differences in regional anthropogenic activities and urban pollution profiles. Despite detectable site-specific variation, the overall elemental concentrations determined in both species were generally low compared with heavily industrialized settings and reference values; however, these preliminary figures must be interpreted within the context of several inherent methodological and biological constraints. Specifically, because carcass collection is opportunistic, demographic covariates (age and sex) in *C. cornix* are unquantified; residual exogenous contamination may be present; and the element panel is limited without concurrent internal tissue or biomarker assessments. Broader conclusions about population-level exposure or toxicological risk cannot

be definitively drawn. Consequently, the feather burdens reported herein should be viewed strictly as descriptive baseline observations rather than definitive indicators of low ecological risk. Overall, these findings underscore the potential utility of opportunistic feather sampling as a complementary preliminary screening tool in urban ecotoxicological surveys, while highlighting the critical need for future research incorporating standardized spatial designs, rural reference populations, and broader contaminant panels to establish rigorous exposure dynamics.

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