



Concentrations of Pb, Cu, and Zn in Feathers of laughing doves (*Spilopelia senegalensis*) as Indicators of Urban Metal Pollution in Iran

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Article Info	ABSTRACT
Article type: Research Article	Heavy metals are among the most important environmental pollutants in urban ecosystems because of their persistence, bioaccumulation potential, and adverse effects on wildlife. Urban-dwelling birds can serve as effective bioindicators of environmental contamination, particularly through the analysis of feathers. This study investigated the concentrations of lead (Pb), copper (Cu), and zinc (Zn) in feathers of laughing doves (<i>Spilopelia senegalensis</i>) collected between January 2021 and January 2022 from three Iranian cities with different levels of urbanization and anthropogenic activity: Sabzevar, Mashhad, and Tehran. Feather samples were obtained from individuals killed in road accidents and subsequently transferred to local environmental departments. After sample preparation and acid digestion, metal concentrations were determined using graphite furnace atomic absorption spectrophotometry (Shimadzu AA 6800). Across the studied cities, Pb concentrations ranged from 0.001 to 0.040 µg/g, Cu from 0.400 to 1.300 µg/g, and Zn from 0.550 to 1.330 µg/g. Significant differences in metal concentrations were observed among the three cities ($p < 0.05$), whereas no significant differences were detected between adult and juvenile individuals. The highest Pb concentrations were recorded in Tehran, while Mashhad exhibited the highest Cu concentrations. Overall, the concentrations of Pb, Cu, and Zn were below reported toxicological threshold levels for birds. These findings suggest that feathers of laughing doves can provide valuable information on the spatial distribution of urban metal pollution and highlight the potential of this species as a practical non-invasive biomonitoring tool for assessing environmental contamination in urban ecosystems of Iran.
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INTRODUCTION

Environmental pollution has increased substantially in recent decades as a result of rapid urbanization, industrial development, population growth, and rising energy consumption. Urban environments are particularly vulnerable to contamination by heavy metals released from vehicular emissions, industrial activities, fossil fuel combustion, waste disposal, and other anthropogenic sources (Gelaye, 2024; Malvandi et al., 2024). Heavy metals are of considerable environmental concern because of their persistence, non-biodegradable nature, and ability to accumulate in biological systems. The United States Environmental Protection Agency (EPA) has identified several metals and metalloids, including lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), zinc (Zn), copper (Cu), nickel (Ni), selenium (Se), and arsenic (As), as priority environmental pollutants due to their ecological and toxicological significance. Although some

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trace elements such as Cu and Zn are essential for normal physiological processes, elevated concentrations may become toxic to living organisms (Malvandi, 2025; Varagiya et al., 2022).

Among these contaminants, Pb is considered one of the most hazardous environmental pollutants because of its high toxicity and widespread occurrence in urban ecosystems. Anthropogenic activities, particularly traffic emissions, industrial processes, fossil fuel combustion, and urban waste, represent major sources of Pb contamination in terrestrial environments (Korbecki et al., 2019; Malvandi and Hassanzadeh, 2018; Malvandi et al., 2021). Exposure to heavy metals may occur through inhalation of contaminated air, ingestion of polluted food and water, or direct contact with contaminated environmental substrates (Malvandi, 2024). Once introduced into biological systems, these pollutants may accumulate in tissues and potentially cause various physiological and ecological effects.

Birds exposed to elevated concentrations of heavy metals may experience a wide range of toxic effects, including reduced reproductive success, behavioral alterations, immunosuppression, oxidative stress, neurological impairment, and tissue damage (Hoynes et al., 2024; Keute et al., 2024; Liu et al., 2015; Sobhan Ardakani and Hosseini, 2024). Chronic exposure to such contaminants may ultimately decrease survival and population fitness, particularly in species inhabiting urban and industrialized environments. Therefore, monitoring heavy metal concentrations in wildlife species is essential for assessing environmental quality and identifying potential ecological risks.

Biomonitoring using living organisms has become an important approach for evaluating environmental contamination, as chemical measurements alone may not fully reflect pollutant bioavailability or biological effects (Liu et al., 2015). Biological indicators provide valuable information regarding pollutant accumulation, exposure pathways, and organismal responses to environmental stressors. Among vertebrates, birds are widely recognized as effective bioindicators because of their broad geographic distribution, ecological diversity, relatively long lifespan, and sensitivity to environmental changes. In addition, birds occupy different trophic levels and may accumulate contaminants through both dietary intake and environmental exposure, making them useful indicators of ecosystem health (Markowski et al., 2013).

Heavy metal accumulation in birds can be evaluated using different tissues and biological matrices, including liver, kidney, muscle, bone, eggs, feces, and feathers (Keute et al., 2024). Among these matrices, feathers have been widely used as a practical and non-invasive biomonitoring tool. Feather analysis offers several advantages, including ease of collection and storage, minimal disturbance to birds, and the possibility of repeated sampling over time (Markowski et al., 2013). Moreover, feathers may reflect both internal metal deposition during feather growth and external contamination from the surrounding environment, thereby providing valuable information about environmental pollution levels (Liu et al., 2015).

Iran hosts more than 500 bird species distributed across diverse ecosystems. Bird species inhabiting urban environments are continuously exposed to anthropogenic pollutants and may therefore serve as useful indicators of urban environmental quality. The laughing dove (*Spilopelia senegalensis*) is one of the most common terrestrial bird species associated with human settlements in Iran. This species is widely distributed in urban and suburban habitats and frequently nests on buildings and other man-made structures. Its close association with human activities, high abundance, and accessibility make it a suitable candidate for biomonitoring studies in urban ecosystems.

Most previous studies investigating heavy metal accumulation in feathers have focused primarily on aquatic or wetland bird species, whereas relatively limited information is available on for terrestrial urban birds, particularly in Iran (Malvandi et al., 2026). Moreover, comparative assessments among Iranian cities with differing levels of urbanization and anthropogenic pressure remain scarce. Accordingly, this study aimed to determine the concentrations of Pb, Cu, and Zn in the feathers of laughing doves collected from Sabzevar, Mashhad, and Tehran, to

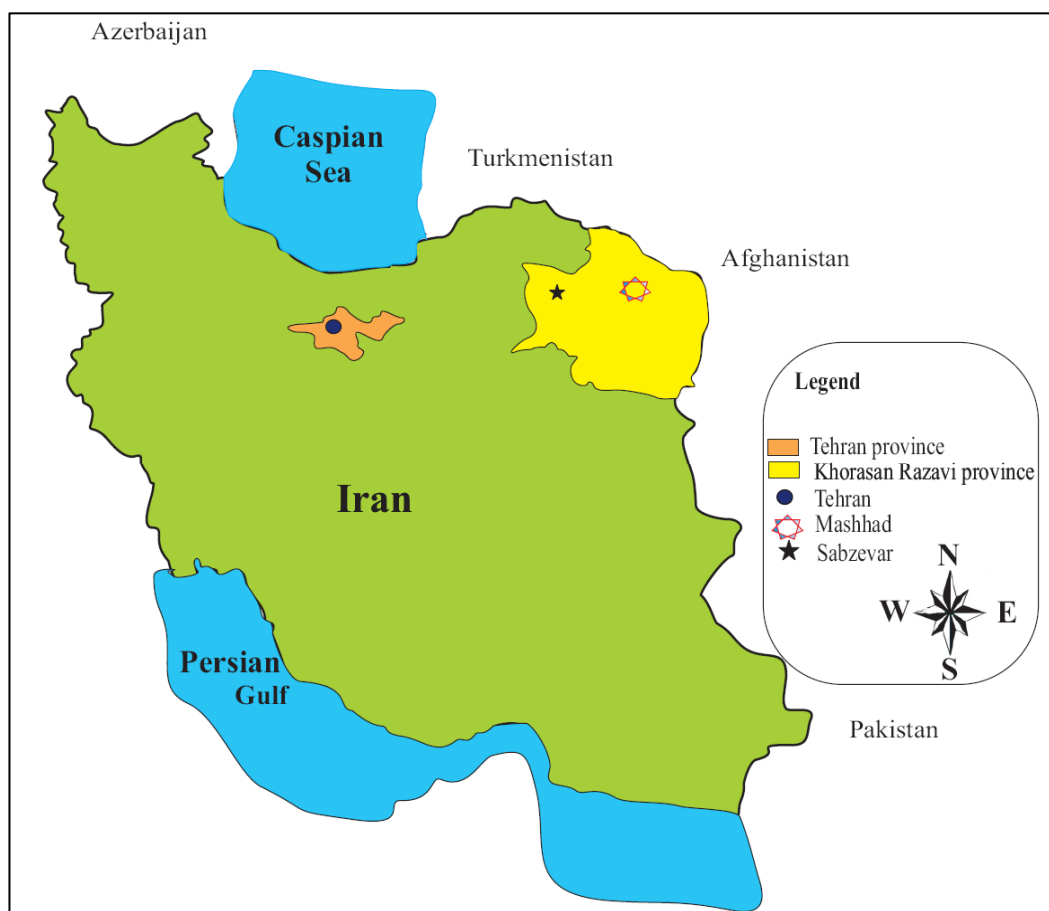


Fig.1. Location of the sampled cities of Tehran, Mashhad and Sabzevar in Iran

evaluate spatial differences in metal concentrations among these urban centers, and to compare the observed concentrations with established toxicological threshold values for birds. We hypothesized that birds inhabiting more densely populated and industrialized cities, particularly Tehran and Mashhad, would exhibit higher feather metal concentrations than those collected from the less urbanized city of Sabzevar.

MATERIALS AND METHODS

Sample collection

Feather samples were collected from laughing doves (*Spilopelia senegalensis*) involved in road accidents, as well as from specimens submitted to the environmental protection departments in Sabzevar, Mashhad, and Tehran, Iran, during a one-year opportunistic survey between January 2021 and January 2022 (Figure 1). No birds were captured or euthanized specifically for the purposes of this study. These cities were selected to represent a gradient of urbanization; Tehran is characterized by high population density and intense industrial activity, while Sabzevar is a smaller urban center with comparatively lower traffic and industrialization.

Feather samples were collected from a total of 42 laughing doves, comprising 18 individuals from Sabzevar, 12 from Mashhad, and 12 from Tehran. No birds were captured or euthanized specifically for this research. Each sample was individually stored in labeled paper envelopes, with the sampling date, location, and age class (adult or juvenile) recorded. Because carcass condition and external morphology did not allow reliable sex determination, molecular sexing

was not feasible; therefore, sex was not included as a factor in the analyses. Tail feathers were specifically selected for analysis to ensure methodological consistency and to minimize variability inherent in different feather types.

Sample preparation and digestion

To reduce exogenous surface contamination, feather samples were washed using a standardized protocol consisting of deionized water, followed by acetone, and a final thorough rinse with deionized water. Because the wash solution/residue was not retained for analysis, the reported concentrations may reflect a combination of internally incorporated metals and any firmly bound surface particulates that were not removed by washing. The cleaned samples were dried in an oven at 60 °C for 24 h and subsequently cut into small fragments using stainless steel scissors.

Approximately 1 g of each dried sample was transferred into acid-washed digestion vessels. Eight milliliters of 65% nitric acid (HNO₃) was added, and the samples were left at room temperature for 12 h for pre-digestion. Subsequently, 2 mL of 60% perchloric acid (HClO₄) was added. The digestion process was conducted in a water bath at 40 °C for 1 h, followed by heating at 140 °C for 3 h. After cooling, the digested solutions were filtered through Whatman No. 42 filter paper and diluted to a final volume of 10 mL using distilled water (López-Perea et al., 2019; Malvandi and Shamabadi, 2022; Malvandi et al., 2010). All concentrations are expressed on a dry weight (dw) basis in µg/g. Elemental analysis was performed using a graphite furnace atomic absorption spectrophotometer (Shimadzu AA-6800).

Quality assurance and quality control

To minimize contamination, all laboratory glassware was soaked in 10% nitric acid for 24 h and rinsed thoroughly with distilled water before use. Quality control measures included the use of procedural blanks, calibration standards, and duplicate analyses. The accuracy of the analytical method was verified using a Certified Reference Material (CRM: BCR-185r, bovine liver). Calibration curves demonstrated high linearity ($R^2 > 0.99$) for all elements. The limits of detection (LOD) ranged from 0.00025 to 0.00098 µg/g, while the limits of quantification (LOQ) ranged from 0.00083 to 0.00329 µg/g. CRM recoveries ranged from 95% to 103%, and relative standard deviations (RSDs) ranged from 5% to 8%, indicating acceptable precision and accuracy of the analytical procedure.

Statistical analysis

Statistical analyses were performed using SPSS software. Data normality was confirmed via the Kolmogorov–Smirnov test, and homogeneity of variances was assessed using Levene's test. Because the data satisfied parametric assumptions, a one-way analysis of variance (ANOVA) was employed to compare metal concentrations among the three cities, followed by Tukey's post hoc test for pairwise comparisons. Independent samples t-tests were used to evaluate differences between age groups (adult vs. juvenile). Pearson correlation analysis was conducted to examine the relationships between the analyzed elements. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Population Structure of Samples and Road Mortality as an Opportunistic Sampling Source

The biological characteristics of each collected specimen, including maturity status and condition at the time of sampling, were recorded. Approximately 33% of the specimens were obtained from road-killed birds. Overall, approximately 60% of the collected individuals were adults and 40% were juveniles. However, sex determination was not possible because the

Table 1. Concentration values of lead, copper and zinc elements ($\mu\text{g/g}$) in the laughing dove species of the studied cities

City	Parameter	Element		
		Pb	Cu	Zn
Sabzevar	Mean	0.007	0.759	0.968
	S. E	0.001	0.039	0.032
	Minimum	0.001	0.400	0.800
	Maximum	0.020	1.100	1.330
Mashhad	Mean	0.011	1.024	1.132
	S. E	0.001	0.034	0.030
	Minimum	0.002	0.900	1.000
	Maximum	0.020	1.300	1.300
Tehran	Mean	0.018	0.850	0.923
	S. E	0.003	0.045	0.061
	Minimum	0.001	0.550	0.550
	Maximum	0.040	1.010	1.200

carcasses lacked distinguishable morphological characteristics.

Road mortality represented an important source of opportunistically collected material analyzed in the present study. Similar observations have been reported in previous investigations conducted in Sabzevar County, where a substantial proportion of bird samples were also obtained from road killed individuals. These included 100% of samples from house sparrow, tree sparrow, crested lark, little owl, Eurasian hoopoe, and European roller, as well as 75% of magpie samples and 10% of little bittern samples (Malvandi and Shamabadi, 2022; Malvandi et al., 2021). Although road mortality is an important wildlife conservation concern, the use of road-killed birds can provide a practical, cost-effective, and ethically acceptable source of biological material for contaminant biomonitoring. Feathers are widely used as a non-destructive matrix for assessing trace metal exposure in birds because metals incorporated into the keratin structure during feather growth can reflect exposure at the time of feather formation (Abdullah et al., 2015; Manjula et al., 2015). Recent studies have also used feathers obtained from carcasses, including birds found on roads or highways, to evaluate heavy metal contamination, supporting the suitability of such material for environmental monitoring when sample condition and collection context are carefully considered (Malvandi and Shamabadi, 2022; Malvandi et al., 2026, 2021; Özdemir and Azizoğlu, 2025). However, because sampling in the present study was opportunistic, the collected individuals may not fully represent the spatial distribution, exposure heterogeneity, or physiological variability of the broader population.

Baseline Feather Concentrations of Pb, Cu, and Zn in Laughing Doves

The concentrations of Pb, Cu, and Zn measured in the feather samples are summarized in Table 1. Lead concentrations ranged from 0.001 to 0.040 $\mu\text{g/g}$ across the study areas, while Cu and Zn concentrations ranged from 0.400 to 1.300 $\mu\text{g/g}$ and 0.550 to 1.330 $\mu\text{g/g}$, respectively. Within the present dataset, Pb concentrations were generally higher in Tehran, whereas Cu and Zn levels were generally higher in Mashhad. Although limited by sample size and sampling design, these data may provide an initial reference point for feather metal concentrations in *Spilopelia senegalensis* from parts of central and northeastern Iran. Because feather metal content is commonly interpreted as an indicator of circulating metal levels during feather formation, the concentrations reported here may reflect, at least in part, environmental exposure during the birds' most recent molting period.

Urban Spatial Patterns of Heavy Metal Accumulation

Statistical analyses revealed significant differences in the concentrations of the studied elements among the three cities. Specifically, Pb levels were significantly higher in Tehran compared to Sabzevar and Mashhad ($F_{2,39}=8.39$, $P=0.001$), while Cu and Zn concentrations were

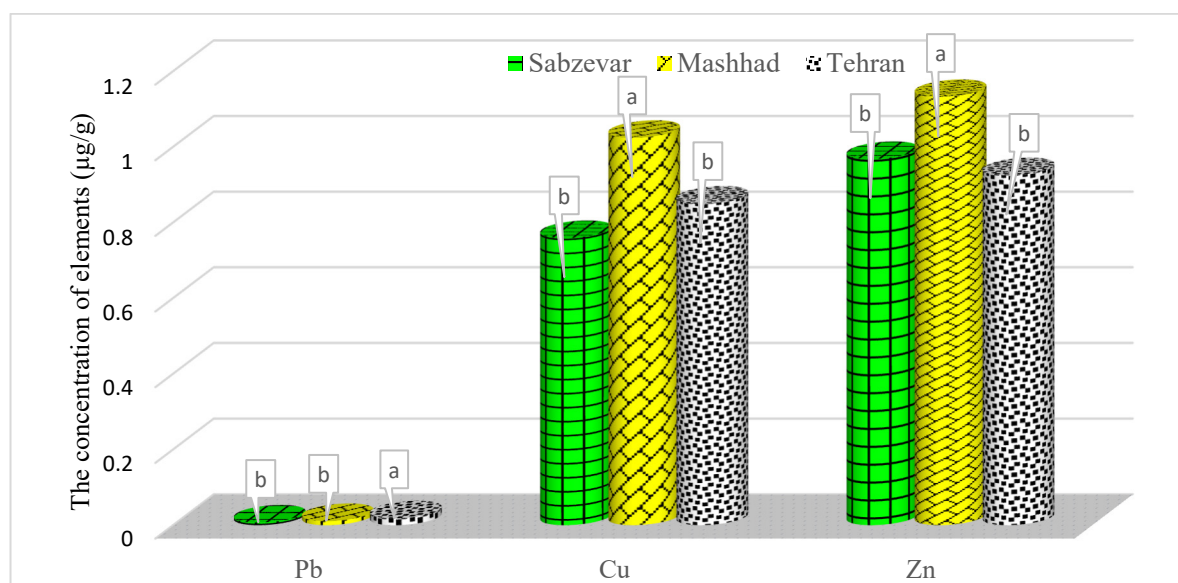


Fig. 2. Mean concentrations ($\mu\text{g/g}$) of Pb, Cu, and Zn in feathers across the three study cities. Different letters above the bars indicate statistically significant differences between locations for each element ($P < 0.05$).

significantly higher in Mashhad ($F_{2,39}=11.34$, $P<0.001$ and $F_{2,39}=6.44$, $P=0.004$, respectively; Figure 2). The elevated Pb levels in Tehran may be associated with the city's intense traffic and vehicle density, which contribute to lead accumulation in urban dust. In addition, regional soil geochemistry and local environment might influence these distribution patterns.

However, because environmental substrates (such as soil or air dust) were not sampled concurrently, these spatial differences reflect comparative trends within our dataset rather than confirmed environmental correlation. Furthermore, metal incorporation is restricted to active feather growth. While *Spilopelia senegalensis* may exhibit prolonged molting cycles in Iran's climate, tail feathers (rectrices) were selected to minimize temporal growth variability. Nevertheless, without direct molt monitoring and environmental validation, distinguishing internal assimilation from external deposition remains challenging. Thus, due to the opportunistic sampling and modest sample size, these patterns represent preliminary site-specific observations rather than national urban trends.

Spatial variation in heavy metal accumulation in birds has been widely documented in previous studies. For example, significant differences in Cd, Pb, and Cr concentrations were reported in wild pigeons (*Columba livia*) from the Brazilian cities of Goiânia and Jataí (Hoff Brait and Antoniosi Filho, 2011). Similarly, a study conducted in Poland reported spatial variation in Pb, Cu, and Zn concentrations in pigeons, with Pb concentrations significantly higher in contaminated sites than in uncontaminated areas (Kurhaluk et al., 2021). In Spain, feather samples of the yellow-legged gull (*Larus michahellis*) were able to distinguish contaminated from non-contaminated areas based on significant differences in Hg, Cd, Pb, and Se concentrations (Vizuete et al., 2022). Comparable spatial patterns of metal accumulation have also been reported for several bird families, including Anatidae, Ardeidae, Sternidae, and Laridae (Korbecki et al., 2019).

Urban environments frequently exhibit higher levels of heavy metal contamination than rural areas. For example, studies on American robin (*Turdus migratorius*), Carolina wren (*Thryothorus ludovicianus*), song sparrow (*Melospiza melodia*), and house sparrow (*Passer domesticus*) in Washington, D.C., reported significantly higher Pb concentrations in birds from urban areas compared with rural sites (Roux and Marra, 2007). Similar findings have been documented for rooks (*Corvus frugilegus*) in Poland (Orłowski et al., 2014), house sparrows in

Palestine (Swaileh and Sansur 2006), and pigeons in Korea (Nam et al., 2004). However, some studies have reported no significant spatial differences in metal concentrations. For instance, Manjula et al. (2015) found no significant variation in Fe, Cu, Zn, and Cd concentrations between urban and rural bird populations in southern India, except for Zn (Manjula et al., 2015). Likewise, Yasmeen et al. (2019) reported no significant differences in metal concentrations in cattle egrets (*Bubulcus ibis*) between rural and urban environments in Pakistan (Yasmeen et al., 2019). These contrasting findings indicate that metal accumulation in birds is influenced by multiple factors, including pollution sources, habitat characteristics, and species-specific ecological traits.

Age-Dependent Trends in Feather Metal Concentrations

In the present study, no significant differences were observed between adult and juvenile individuals in the concentrations of Pb, Cu, and Zn ($P > 0.05$). This suggests that, within our limited dataset, age was not a primary driver of metal accumulation in the feathers of the sampled laughing doves. A total of 25 adults and 17 juveniles were included in this comparison. Mean Pb concentrations were 0.013 ± 0.008 $\mu\text{g/g}$ in adults and 0.009 ± 0.008 $\mu\text{g/g}$ in juveniles. Mean Cu concentrations were 0.869 ± 0.218 and 0.848 ± 0.123 $\mu\text{g/g}$, while Zn concentrations averaged 0.989 ± 0.164 and 1.020 ± 0.185 $\mu\text{g/g}$ for adults and juveniles, respectively.

Although adult birds are generally expected to accumulate higher concentrations of metals due to longer exposure periods and potentially broader foraging ranges, several studies have similarly reported no significant age related differences. Such findings have been documented in species including the black swan (*Cygnus atratus*) in Australia (Nzabanita et al., 2024), (Sheybanifar et al., 2016), the pink gull (*Larus genei*) in the Persian Gulf (Hashemi et al., 2017), and the great tit (*Parus major*) in the Czech Republic (Bauerová et al., 2020). In contrast, other studies have reported significant age related differences. For example, higher Cd concentrations were found in adult crows compared with juveniles in Hungary (Bauerová et al., 2020), while elevated Hg, Cd, Pb, and Se concentrations were observed in adult yellow legged gulls compared with younger individuals (Vizuet et al., 2022). These discrepancies may reflect differences in feeding ecology, exposure pathways, and species specific physiological characteristics. In addition, because some samples were obtained opportunistically from carcasses, variation in carcass condition or the interval between death and collection may have introduced an additional source of uncertainty, although the keratinized structure of feathers is generally considered relatively stable for metal assessment. Because the age-based comparison was conducted on a relatively limited number of individuals, and molecular sexing was not feasible due to the opportunistic nature of the sampling, these results should be interpreted as preliminary.

Potential Influence of Sex on Metal Bioaccumulation

Because the collected carcasses lacked reliable morphological traits for sex identification and no molecular sexing was performed, sex-related differences in feather metal concentrations could not be evaluated in the present study. This limitation should be considered when interpreting the observed inter-individual variability, as sex may influence metal accumulation through differences in exposure patterns, physiology, or reproductive processes. For example, the transfer of certain metals to eggs during reproduction may contribute to lower concentrations in females. Therefore, although the present findings provide useful preliminary baseline data, they do not allow determination of whether sex played a role in the metal burden observed in these urban birds.

Ecotoxicological Implications of Detected Metal Levels

The potential ecological and physiological risks associated with the detected metal concentrations were evaluated by comparing the measured values with previously reported

toxicity thresholds. Lead is a non-essential and highly toxic element that can adversely affect several physiological systems in birds, including reproduction, growth, neurological function, and thermoregulation. Feather concentrations exceeding approximately 0.4 µg/g have been associated with adverse biological effects (Abdullah et al., 2015; Picone et al., 2019; Schmude et al., 2018; Yamac et al., 2019). In the present study, Pb concentrations in all analyzed samples were well below this threshold, suggesting no evidence of acute Pb toxicity in the sampled individuals. Similarly, Cu and Zn concentrations remained within non-toxic ranges and were far below levels typically associated with avian toxicity (Abdullah et al., 2015; Manjula et al., 2015; Zarrintab and Mirzaei, 2018).

Whether these low concentrations reflect species-specific physiological handling, including metal sequestration and excretion via feather growth, or a lower-than-expected environmental bioavailability of metals in these urban areas remains unresolved. Therefore, the observed concentrations should be interpreted cautiously and primarily as city-specific baseline exposure values rather than indicators of maximum contamination. Because the samples were collected opportunistically, they may preferentially represent birds from relatively cleaner suburban or urban green micro-habitats rather than from the most heavily polluted industrial zones. Finally, while the washing protocol was designed to remove loosely bound exogenous particles, the absence of a washing residue analysis means that these values may still reflect a combination of internally incorporated metals and firmly bound atmospheric particulates.

Elemental Associations and Comparative Context with Global Avian Studies

Correlation analysis revealed no significant relationships among Pb, Cu, and Zn concentrations in the analyzed feather samples. The absence of significant correlations suggests that these elements may originate from different environmental sources or exposure pathways. Similar patterns have been reported in other studies; for example, Liu et al. (2015) observed a significant correlation between Cu and Zn but not between Pb and the other examined elements in several waterbird species.

Finally, Table 2 compares the concentrations of Pb, Cu, and Zn measured in the present study with values reported for other bird species worldwide. Overall, the concentrations observed in laughing doves were generally lower than those reported in many previous studies. Notably, the only available study on laughing doves in Iran, conducted in Yazd, reported substantially higher Pb concentrations (Akhavan Ghalibaf et al., 2017). This discrepancy could be attributed to several factors. Beyond differences in regional industrial profiles and analytical methodologies, the opportunistic nature of our sampling must be considered. In Yazd, samples might have been collected from core industrial or high-exposure zones, whereas our opportunistic carcasses in Tehran, Mashhad, and Sabzevar may have originated from suburban areas, parks, or residential pockets with lower localized heavy metal emissions. Because we could not standardize the exact home ranges of the salvaged birds prior to their death, these spatial comparisons remain exploratory.

Study Limitations

While this study provides valuable baseline data for trace metal accumulation in laughing doves, several limitations must be acknowledged. First, the relatively modest sample size and unequal distribution across cities restrict the statistical power of the analyses and the ability to generalize these findings nationwide. Second, the opportunistic sampling of road-killed or salvaged carcasses introduces potential spatial and physiological bias, as the exact foraging range, pre-mortem health status, and exposure history of the birds prior to death could not be determined. Third, the opportunistic collection occurred over a one-year survey rather than simultaneously across all regions, meaning that seasonal variations in feather growth and metal deposition could not be systematically controlled. Fourth, due to the degraded state of many

Table 2. Average concentration of lead, zinc and copper (µg/g) in feather samples from different birds from different parts of the world.

Common name	Scientific name	Element			Location	Reference
		Pb	Cu	Zn		
Laughing dove	<i>Spilopelia senegalensis</i> ^a	0.007	0.759	0.968	Sabzevar, Iran	Present study
Laughing dove	<i>Spilopelia senegalensis</i> ^a	0.011	1.024	1.131	Mashhad, Iran	Present study
Laughing dove	<i>Spilopelia senegalensis</i> ^a	0.018	0.850	0.922	Tehran, Iran	Present study
Laughing dove	<i>Spilopelia senegalensis</i> ^b	19.88	N. A ^c	N. A	Yazd, Iran	(Akhavan Ghalibaf et al., 2017)
Feral pigeon	<i>Columba livia</i> ^b	204.80	13.15	13.82	Paris, France	(Frantz et al., 2012)
Feral pigeon	<i>Columba livia</i> ^a	3.22	N. A	N. A	Goiânia and Jataí, Brazil	(Hoff Brait and Antoniosi Filho, 2011)
Feral pigeon	<i>Columba livia</i> ^b	5.70	N. A	N. A	Korea	(Nam et al., 2004)
Feral pigeon	<i>Columba livia</i> ^b	2.88	5.06	48.195	Poland	(Kurhaluk et al., 2021)
Feral pigeon	<i>Columba livia</i> ^b	0.310	N. A	N. A	Harbin, Beijing, and Guangzhou, China	(Cui et al., 2021)
Spotted dove	<i>Streptopelia chinensis</i> ^b	2.83	8.31	79.82	China	(Yao et al., 2023)
Tree sparrow	<i>Passer montanus</i> ^b	7.05	27.60	152.67	China	(Yao et al., 2023)
Common Blackbird	<i>Turdus merula</i> ^b	3.41	13.28	91.04	China	(Yao et al., 2023)
Yellow-legged gulls	<i>Larus michahellis</i> ^a	4.38	N. A	N. A	Spain	(Vizuete et al., 2022)

^a µg/g dw; ^b µg/g; ^c N.A: Non available

collected carcasses, molecular sexing was not feasible, preventing the evaluation of sex-specific bioaccumulation pathways. Fifth, the lack of both washing residue analysis and concurrent environmental monitoring (such as ambient air particulate matter and soil sampling) limits our ability to quantitatively differentiate between internal systemic absorption and external atmospheric deposition on the feathers. Future studies employing larger, systematically randomized sample sizes and integrating co-located environmental samples (dust and soil) are required to address these limitations and validate the species' long-term biomonitoring efficacy.

CONCLUSION

This study evaluated the concentrations of lead (Pb), copper (Cu), and zinc (Zn) in the feathers of laughing doves (*Spilopelia senegalensis*) collected from three Iranian cities: Sabzevar, Mashhad, and Tehran. The results indicated no significant differences in metal concentrations between adult and juvenile individuals. However, significant spatial differences were detected among the studied cities, suggesting the influence of local environmental conditions and potential pollution sources. The health risk assessment demonstrated that the concentrations of Pb, Cu, and Zn measured in all samples were well below levels reported to cause adverse effects in birds. Therefore, these elements are unlikely to pose a significant health risk to the studied populations of *S. senegalensis*, even in highly urbanized environments such as Tehran. Because the study was based on a relatively small number of opportunistically collected samples, the findings should be interpreted as preliminary comparative evidence for the three investigated cities rather than as a basis for nationwide conclusions about urban pollution in Iran. Overall, the findings highlight the value of feather analysis in urban birds as a non-invasive biomonitoring tool for assessing environmental contamination. Such investigations provide useful insights

into heavy metal pollution in urban ecosystems and help improve understanding of wildlife exposure in environments heavily influenced by human activities. Future studies incorporating larger and more balanced sample sizes, additional cities, and multiple bird species are needed to confirm the observed patterns and to strengthen broader biomonitoring inferences in Iranian urban environments.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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