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Assessment of Metal Contamination in the Kufa River Environment Using Waterhen (*Gallinula chloropus*) Feathers as a Potential Biomonitoring Tool

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ABSTRACT

Here we present summary data for the concentrations of eight elements – lead (Pb), copper (Cu), nickel (Ni), cadmium (Cd), zinc (Zn), iron (Fe), chromium (Cr) and manganese (Mn) – measured in waterhen (Gallinula chloropus) feathers by atomic absorption spectrometry (AAS) in the Kufa River environment, Najaf Governorate, Iraq. The values provided for the present analysis reflect ten independent waterhen samples, 2/mo. Jan-May, for each element, for a total of 20 individual samples. Condition-specific assumed conversion from milli-grams per litre (mg/L) to milli-grams per kilogram (mg/kg) dry body weight was based on a 0.75g sample and a 25mL final digest volume, assuming no dilution was introduced between digestion and analysis, and subtraction of method blank absorbances is appropriate. The highest mean concentrations of elements at each collection site were recorded for zinc and iron (68.33 mg/kg), while concentrations of cadmium were lowest (0.05-0.17 mg/kg). Mean concentrations of several elements at each collection site were lower in months of March and May than in January, February, and/or April; cadmium had the highest mean in April. Exact permutation Kruskal-Wallis p-values determined without correction for multiple comparisons were lower than 0.05 for a number of elements, but if Holm correction is applied for multiple comparisons across 8 elements none had p 0.076. The data are exploratory because there were only 2 independent samples per month, and because the assumptions leading to dry weight conversions in mg/kg are unverified; the results are descriptive of the current ten-sample dataset only, not an experimental assessment of river quality.

KEYWORDS: Gallinula chloropus; waterhen; feathers; heavy metals; atomic absorption spectrometry; Kufa River; Iraq; biomonitoring

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1. INTRODUCTION

Metals originate from both natural and anthropogenic sources in riverine and wetland ecosystems - urban effluent, agricultural runoff, industrial emission, solid waste disposal, and removal of metal-rich sediments. Cu, Fe, Mn, and Zn are critical nutrients at suitable concentrations, but may be harmful at high exposures; Pb and Cd are considered toxic; but the implication of a particular value, depends on the ligand chemistry, the way it is expressed, and the organism.

Waterbirds have the potential to integrate contaminant exposure across aquatic food webs, as they both contact water and sediment, and could potentially consume aquatic plants and invertebrates. The most common moorhen/waterhen (Gallinula chloropus) is associated with wetlands and may make a good candidate species for biomonitoring, especially as feathers are a convenient sampling material, though the mode of release of metals into the feathers is not entirely clear (incorporation during feather formation, or external deposition from water, dust or sediment). Differences between feather type, cleaning, molting and analytical QC can all influence the outcome (Rutkowska et al., 2018; Jaspers et al., 2019; Dauwe et al., 2003).

Samples of G. chloropus from southern Iraq have previously been sampled for metal analysis in feathers and other tissues, allowing a geographically appropriate comparison (Abdul Jaleel, 2020). Other investigations of different avian species have used feathers as the matrix to examine environmental contamination of metals (Aziz et al., 2021; Khwankitrittikul et al., 2024). Numerical comparisons must be based on consistent tissue matrices, preparation, reporting basis, and analytical techniques. The current study presents the compilation of AAS analyses of Pb, Cu, Ni, Cd, Zn, Fe, Cr, and Mn in sample related to the environment of the Kufa River. The aims of this study were to record the mean of monthly concentrations, to generate, with the sample weight and digest volume set as conditions, dry-weight data using the actual weight of sample as mean for each month and to characterize change by month and on compare month to month differences with (obviously tiny number of samples by months).

2. MATERIALS AND METHODS

2.1. Study Area

The sampling activity was carried out in the Kufa River (Shatt al-Kufa), in the Kufa District/ Governorate of Najaf, Iraq. Kufa is situated on the east of the Shatt al-Kufa (Euphrates River), about 10 km northeast of Najaf City. The area has a semi-arid climate, with hot summers and moderate winters, and consists of urban and rural areas. (Rashed et al., 2026).

The Kufa River is a significant local water ecosystem affected by land-use activities. The distribution of trace metals in its bottom sediments has been previously studied, which indicates the importance of environmental monitoring in this river (Jasim, 2025). In another work, the residue of pesticides has been analyzed in the river and ecotoxicological influences of the runoff and wastewater discharges were reported (Mohammed & Al-Haidarey, 2025). The Kufa River region is, thus, an ideal ecosystem to monitor the metal concentrations in the feathers of waterhen (Gallinula chloropus) as a bio-monitoring material. For this purpose, three feather samples from the study area were used to analyze Pb, Cu, Ni, Cd, Zn, Fe, Cr and Mn. To determine the metals concentrations by AAS.

2.2. Sample Collection and Preparation

Of the common moorhen (Gallinula chloropus) specimens, 35 feathers were removed from the Kufa River setting. For the purpose of evaluating the content of various metals and the bioconcentration potential of waterhen feathers as an environmental biomonitoring tool for environmental metal pollution, the samples were prepared and a 0.75-g sample was weighed and inserted into a digestion vessel (Dauwe et al., 2003; Aloupi et al., 2020).

2.3 Feather Cleaning and Drying

Feather samples were handled individually to avoid cross contamination during sample preparation. Before chemical analysis, the samples were washed with the washing procedure followed in the laboratory to remove any dust and other external impurities adhered to the feathers. This is necessary since surface-associated metals may have an effect on the concentrations measured and thus on the interpretation of the results of feather-based biomonitoring (Dauwe et al. 2003, Aloupi et al. 2020). The washing agents used were 10% HNO3. After the washing, the samples were dried at 60°C for 24 hours until the preparation procedure was completed and the prepared samples were weighed prior to digestion.

2.4 Acid Digestion

Sample preparation for chemical digestion 0.75 g of each sample of feather was weighed and placed in a dry digestor. All the samples were digested using Teflon tubes containing 6 ml of 65% GR-grade HNO3 with a graphite digestion system, with a total volume of 25 ml after dilution. Samples were digested at 60 °C for 24 h. The digest solutions were cooled and filtered, if necessary, prior to instrumental analysis. The digest solutions were used for the determination of the target elements.

2.5 Determination of Heavy Metals by Atomic Absorption Spectroscopy

Concentrations of Pb, Cu, Ni, Cd, Zn, Fe, Cr, and Mn were analyzed with atomic absorption spectroscopy (AAS). Analysis was conducted with an AAS instrument (Perkin Elmer, Waltham, MA, USA, model PinAAcle 900T) and calibration was performed using the appropriate standard solutions for the element of interest. Reagent blanks and calibration checks were prepared to determine contamination of samples and to verify instrument accuracy.

The digest solutions were used to determine the concentration of each metal present in the feathers by adjusting for the final volume of digestion, mass of the sample analysed and any additional dilution factor applied to the digest solution. Results are expressed in mg.kg^-1 dry weight. Dry weight if the sample weight is expressed on a dry weight basis and calculation based on blankcorrected values.

2.6 Quality Assurance and Quality Control

The following must be done to assess for possible contamination, calibration drift, analytical accuracy, and method precision; A procedural reagent blank must be prepared with the reagents used to digest the sample and it should be run simultaneously with the sample to check for contamination. Element specific calibration graphs, utilizing appropriate analytical standard solutions, should

be produced with a correlation coefficient (r) of no less than 0.995 as the proposed calibration acceptance criteria which is comparable to other atomic absorption spectrophotometry guidance (U.S. Environmental Protection Agency, 2007).

2.7 Statistical Analysis

For each element, the mean ± sample SD was calculated from the two monthly values of each element (eight samples in total), but due to the very small sample size, normality tests and parametric comparisons were not of use. The effects of month were analysed using an exact permutation version of the Kruskal-Wallis rank test. The tie-corrected H statistic value measured on the original data set was compared with a distribution obtained by all possible reallocation of the ten observations among five labelled groups of two (113,400 arrangements for each element). P-values were corrected for the group of eight element specific tests using the Holm procedure. Percentage change between January and May was derived from the mean monthly values as follows: (May – January)/January*100. Because n = 2 per month, all results are considered to be exploratory.

3. RESULTS

3.1. Individual AAS readings

The individual digest readings supplied for the ten samples are presented in Tables 1a and 1b. All readings were retained as provided, including the February values initially labelled "Limit," which the researcher confirmed are analytical readings. The two observations per month were treated as independent feather samples. Results are reported in mg/L for the digest solutions.

Table 1a. Individual AAS readings for Pb, Cu, Ni, and Cd (mg/L).

Month	Sample ID	Pb	Cu	Ni	Cd
January	Jan-1	0.2	0.25	0.21	0.004
January	Jan-2	0.1	0.19	0.2	0.001
February	Feb-1	0.1	0.2	0.23	0.001
February	Feb-2	0.12	0.21	0.19	0.002
March	Mar-1	0.01	0.05	0.02	0.003
March	Mar-2	0.02	0.04	0.03	0.004
April	Apr-1	0.02	0.2	0.1	0.006
April	Apr-2	0.03	0.21	0.2	0.004
May	May-1	0.01	0.05	0.02	0.003
May	May-2	0.01	0.04	0.02	0.004

Table 1b. Individual AAS readings for Zn, Fe, Cr, and Mn (mg/L).

Month	Sample ID	Zn	Fe	Cr	Mn
January	Jan-1	1.7	1.8	0.22	0.43
January	Jan-2	2	2	0.15	0.51
February	Feb-1	2.1	2	0.11	0.45
February	Feb-2	2	2.1	0.12	0.4
March	Mar-1	0.5	0.3	0.05	0.1
March	Mar-2	0.41	0.27	0.034	0.1
April	Apr-1	2	2	0.1	0.5
April	Apr-2	1.9	2.1	0.11	0.5
May	May-1	0.5	0.3	0.05	0.1
May	May-2	0.4	0.2	0.04	0.21

Note: R1 and R2 correspond to two separate feather samples per month, not repeated instrument readings of a single digest.

3.2. Monthly mean digest concentrations

Monthly mean digest concentrations varied by element (Table 2). The highest monthly mean for Pb and Cr occurred in January (0.150 and 0.185 mg/L, respectively), whereas Cu peaked in January (0.220 mg/L) and Ni in February (0.210 mg/L). Cd reached its highest mean in April (0.0050 mg/L). Zn peaked in February at 2.050 mg/L, while Fe reached 2.050 mg/L in both February and April; Mn peaked in April at 0.500 mg/L.

Table 2. Monthly mean AAS digest concentrations (mean ± SD, mg/L; n = 2 independent samples per month).

Element	Jan	Feb	Mar	Apr	May
Lead (Pb)	0.15 ± 0.0707	0.11 ± 0.0141	0.015 ± 0.00707	0.025 ± 0.00707	0.01 ± 0
Copper (Cu)	0.22 ± 0.0424	0.205 ± 0.00707	0.045 ± 0.00707	0.205 ± 0.00707	0.045 ± 0.00707
Nickel (Ni)	0.205 ± 0.00707	0.21 ± 0.0283	0.025 ± 0.00707	0.15 ± 0.0707	0.02 ± 0
Cadmium (Cd)	0.0025 ± 0.00212	0.0015 ± 0.000707	0.0035 ± 0.000707	0.005 ± 0.00141	0.0035 ± 0.000707
Zinc (Zn)	1.85 ± 0.212	2.05 ± 0.0707	0.455 ± 0.0636	1.95 ± 0.0707	0.45 ± 0.0707
Iron (Fe)	1.9 ± 0.141	2.05 ± 0.0707	0.285 ± 0.0212	2.05 ± 0.0707	0.25 ± 0.0707
Chromium (Cr)	0.185 ± 0.0495	0.115 ± 0.00707	0.042 ± 0.0113	0.105 ± 0.00707	0.045 ± 0.00707
Manganese (Mn)	0.47 ± 0.0566	0.425 ± 0.0354	0.1 ± 0	0.5 ± 0	0.155 ± 0.0778

3.3. Conditional dry-weight concentrations in feathers

After conversion using the stated sample mass and final digest volume, Zn and Fe yielded the highest monthly mean concentrations, reaching 68.33 mg/kg dry weight in February for both elements and also in April for Fe. Monthly mean Zn was 65.00 mg/kg in April. Mn ranged from 3.33 mg/kg in March to 16.67 mg/kg in April. The lowest mean concentrations were observed for Cd, ranging from 0.050 to 0.167 mg/kg. These values are conditional on DF = 1, a dry sample mass of 0.75 g for each sample, a final digest volume of 25 mL, and appropriate blank correction.

Table 3. Conditional monthly mean concentrations in feather samples (mean ± SD, mg/kg dry weight; calculated with DF = 1).

Element	Jan	Feb	Mar	Apr	May
Lead (Pb)	5 ± 2.36	3.67 ± 0.471	0.5 ± 0.236	0.833 ± 0.236	0.333 ± 0
Copper (Cu)	7.33 ± 1.41	6.83 ± 0.236	1.5 ± 0.236	6.83 ± 0.236	1.5 ± 0.236
Nickel (Ni)	6.83 ± 0.236	7 ± 0.943	0.833 ± 0.236	5 ± 2.36	0.667 ± 0
Cadmium (Cd)	0.0833 ± 0.0707	0.05 ± 0.0236	0.117 ± 0.0236	0.167 ± 0.0471	0.117 ± 0.0236
Zinc (Zn)	61.7 ± 7.07	68.3 ± 2.36	15.2 ± 2.12	65 ± 2.36	15 ± 2.36
Iron (Fe)	63.3 ± 4.71	68.3 ± 2.36	9.5 ± 0.707	68.3 ± 2.36	8.33 ± 2.36
Chromium (Cr)	6.17 ± 1.65	3.83 ± 0.236	1.4 ± 0.377	3.5 ± 0.236	1.5 ± 0.236
Manganese (Mn)	15.7 ± 1.89	14.2 ± 1.18	3.33 ± 0	16.7 ± 0	5.17 ± 2.59

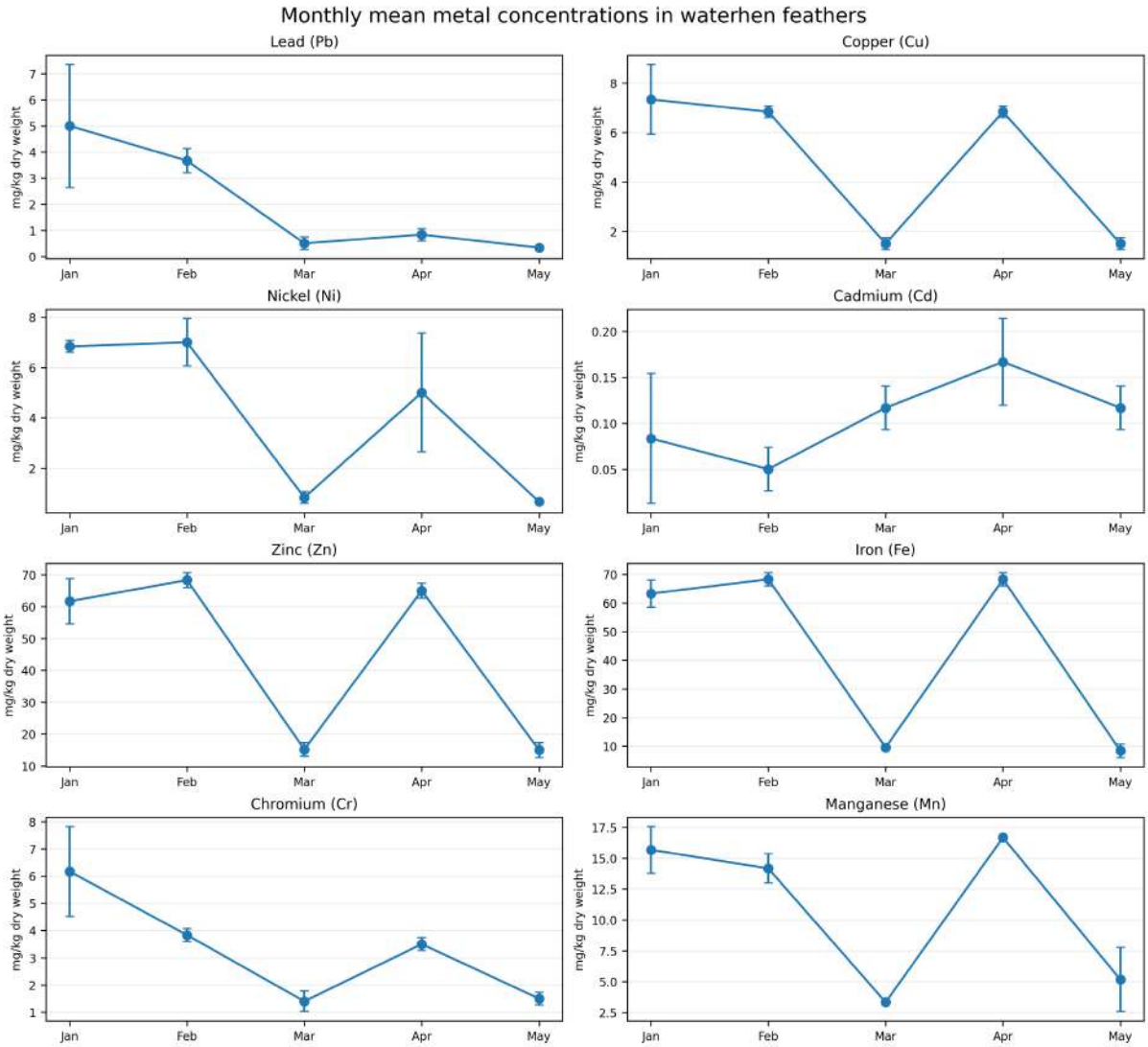


Figure 1. Monthly mean concentrations of the eight elements in feather samples, expressed as mg/kg dry weight. Error bars represent ±1 sample SD (n = 2 per month). Values are conditional calculations based on a 0.75-g dry sample and a 25-mL final digest volume, with DF = 1.

3.4. Relative change from January to May

Comparing the January and May monthly means, seven elements showed lower means in May, while Cd increased by 40.0%. Pb showed the largest percentage decrease (93.3%), followed by Ni (90.2%) and Fe (86.8%). The calculated percentage changes are descriptive comparisons between two months and do not indicate a statistically established trend over time.

Table 4. Percentage change in monthly mean digest concentrations between January and May.

Element	January mean (mg/L)	May mean (mg/L)	Change
Lead (Pb)	0.15	0.01	-93.3%
Copper (Cu)	0.22	0.045	-79.5%
Nickel (Ni)	0.205	0.02	-90.2%
Cadmium (Cd)	0.0025	0.0035	+40.0%
Zinc (Zn)	1.85	0.45	-75.7%
Iron (Fe)	1.9	0.25	-86.8%
Chromium (Cr)	0.185	0.045	-75.7%
Manganese (Mn)	0.47	0.155	-67.0%

3.5. Exact permutation tests across months

The exact permutation Kruskal–Wallis tests showed unadjusted p-values below 0.05 for Pb, Zn, Fe, Cr, and Mn. However, none of the eight element-wise tests remained statistically significant after Holm correction (adjusted p-values ranged from 0.076 to 0.314; Table 5). Therefore, the analysis did not provide multiplicity-adjusted statistical evidence of monthly differences at $\alpha = 0.05$. Given the sample size of only two observations per month, the results should be regarded as preliminary and low-powered.

Table 5. Exact permutation Kruskal–Wallis test results comparing five months for each element (n = 2 per month).

Element	H (df = 4)	Exact p	Holm-adjusted p	Interpretation (α = 0.05)
Lead (Pb)	8.038	0.0222	0.1556	Not significant after Holm correction
Copper (Cu)	6.708	0.1196	0.2392	Not significant after Holm correction
Nickel (Ni)	7.397	0.0603	0.2095	Not significant after Holm correction
Cadmium (Cd)	5.324	0.3143	0.3143	Not significant after Holm correction
Zinc (Zn)	7.566	0.0413	0.2095	Not significant after Holm correction
Iron (Fe)	7.585	0.0349	0.2095	Not significant after Holm correction
Chromium (Cr)	8.227	0.0095	0.0762	Not significant after Holm correction
Manganese (Mn)	7.650	0.0413	0.2095	Not significant after Holm correction

The exact p-values were obtained by enumerating all 113,400 allocations of the ten observations to five month groups of two. Holm correction was applied across the eight element-wise tests.

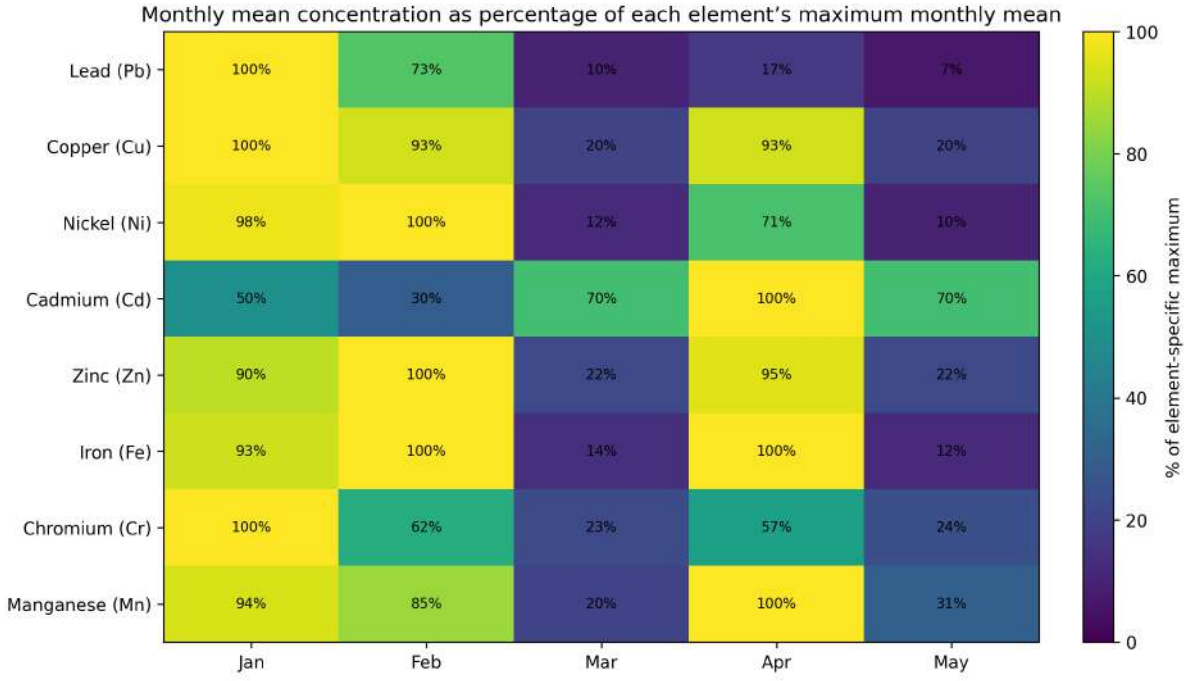


Figure 2. Heatmap showing each element's monthly mean concentration as a percentage of that element's maximum monthly mean. Normalization is performed separately for each element and is intended to compare temporal patterns, not absolute concentrations among different elements.

4. DISCUSSION

The distribution of the five month data set of the eight elements showed a varied temporal pattern: Pb, Cu, Zn, Fe, Cr and Mn showed higher values in some or all of the months of January, February and April while their lowest values were generally recorded for March and May. Ni was high in January and February then decreased in March, increased in April and then decreased again in May. In contrast, Cd showed a different time series with high values in April. This implies that the map of elements varies.

The steep drop and rise in a number of the monthly means should not be simply assumed to indicate that the level of environmental contamination in the Kufa River had fallen and then risen. Feather concentrations may represent both the time relative to feather growth and time of exposure before feather collection (Feather deposition) as well as external contamination on the surface of the feather. Variations among months may also reflect heterogeneity among samples, feather types, type and duration of exposure, analytical variability or other variables not measured in this dataset (Dauwe et al., 2003; Aloupi et al., 2020). This dataset spans only January to May and two samples per month, so a recurring seasonal pattern cannot be identified, let alone an explanation for it.

The top ranked (in order of highest to lowest calculated concentrations in the monthly summary tables) was Zn and Fe, then Mn. This describes the data in these tables (not whether the metals are toxic). Zn, Fe, Cu and Mn are essential elements, with effects that are dependent on extent and form of exposure, and we cannot infer adverse effect based on the concentration in feathers alone. Pb and Cd occurred at lower concentrations than Zn or Fe for the present dataset but we cannot interpret their environmental or biological significance based on the rank of concentrations.

This interpretation is in keeping with the wider literature, which demonstrates that metals in feathers are element, tissue, species and sampling condition specific (Aziz et al., 2021; Khwankitrittikul et al., 2024). Several studies have used bird feathers to monitor selected toxic metals (Aziz et al., 2021). Khwankitrittikul et al. (2024) also highlighted the importance of knowing whether concentration patterns differ among tissues and species. A comparative analysis with a regional G. chloropus (from the south of Iraq) may be more valid; however, any numerical comparison with G. chloropus figures in the literature relies on the specific feather type and digestion procedure used, the analytical technique, and the metric presented (Abdul Jaleel, 2020). The values reported are generated from solution readings in mg/L and cannot be directly compared with published feather values in mg/kg unless the mass-volume conversion factor and dilution factor are checked.

Feathers are suited to minimal-impact or non-invasive monitoring, but don't offer a simple direct measure of contemporaneous river water contamination. Metals can be incorporated into growing feathers from circulating blood, while metals coming from dust, water and sediment can stay on feather surfaces. Dauwe et al. (2003) reported potential effects of molt and external contamination on feather metal levels, and Aloupi et al. (2020) specifically examined feather decontamination procedures. These considerations make the specific feather collection and cleaning protocols significant when comparing study results.

No paired water or sediment samples, no tracers of metal sources and no biological-effect data are available in the current dataset. So the data are useful for detecting patterns in metals measured in feathers but cannot identify the source of any metal, quantify the overall degree of river contamination or demonstrate ecological harm. Future monitoring would benefit from paired water, sediment and feather samples at predefined sites and dates, along with full method blanks and appropriate reference materials.

6. CONCLUSIONS

The ten sample-level AAS records for January-May indicated that all eleven monthly mean values of Zn and Fe (after conditional conversion to dry-weight concentrations) were higher than those of any other element. The shape of the temporal patterns was not consistent among elements, with a few metals lower in May and March, but with Cd peaking in April. Nonparametric permutation Kruskal-Wallis tests did not find any statistically significant month effects after pre-hoc Holm correction of p-values across the eight elements. However, these interpretations are tentative, as only two independent feather samples were used in each month, conversion to mg/kg was based on an assumed dry weight mass of 0.75 g, a 25-mL final digest volume with no further dilution, and a blank correction. The entire inventory of samples, lab protocol, and records of lab analytical quality-control should be further reviewed, and all possible samples should be tested before conclusions are drawn about metal levels in the Kufa River environment.

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