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# Biomonitoring of heavy metals in the feathers raptors from eastern Anatolia of Türkiye

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This study aimed to determine the levels of heavy metal(loid) in the primary, secondary and breast feathers of carcasses of raptor species detected on highways within the borders of Van and Hakkari Provinces. The study was planned to take place between 1 April 2021 and 30 May 2022, including the spring and autumn migration periods. The study stations were selected considering the routes where birds breed and are frequently active. In addition, observations were made along the way, and the amounts of As, Cd, Cr, Cu, Ni, Pb, and Zn in the primary and secondary wing feathers and breast feathers of 19 carcasses belonging to 11 species collected from 3 families (Accipitridae, Falconidae, Strigidae) belonging to two raptor orders (Accipitriformes, Strigiformes) were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). The metal(loid) with the highest accumulation rates in the carcass feathers were ranked as follows: Zn > Cu > Cr > Ni > Pb > As > Cd. Cd values were not found in the Strigidae and Falconidae families. It was only read in the family Accipitridae. There is a significant difference in the average Cu metal level between the Falconidae and Accipitridae families. When As, Ni, Cr and Pb evaluated, no difference was seen between the species groups ($p>0.05$). There was a difference between Zn and Cd, but no difference was observed for the other elements ($p<0.05$). According to the detected concentrations of these metals, it was seen that the levels of accumulation in the feathers of the examined birds varied depending on their feeding habits.

KEYWORDS

**Accipitriformes, birds feathers, ecotoxicology, metal(loids) pollution, Strigiformes**

## 1 Introduction

In recent years, there has been mounting concern regarding the impact of toxic metals on human, animal and environmental welfare (Grúz et al., 2019; Iqbal et al., 2023). This is due to the pollution arising from increased intensity of industrial, commercial, mining and agricultural production (Napa et al., 2015; Das et al., 2023). Heavy metals are defined as natural compounds that are found in low concentrations in the environment. They are constantly released into the environment from natural sources, such as volcanic activity or rock weathering (Cimboláková et al., 2019; Briffa et al., 2020: Masindi et al., 2021).

Additionally, industrial processes and some agricultural activities have greatly increased the transport of many metals into freshwater ecosystems (Tchounwou et al., 2012). For this reason, In recent years, the issue of heavy metal pollution has assumed significant importance (Munzuroğlu and Gür, 2000; Timothy and Williams, 2019). These metals are defined as persistent and non-biodegradable substances. They have the capacity to bioaccumulate within living organisms. Many organisms are potentially harmed by some level of exposure to these metals (Güven, 1999). Heavy metals are largely released into the environment through industrial waste, organic waste, vehicle exhaust fumes, garbage burning and energy production (Xia et al., 2011; Markowski et al., 2013; Ilyas et al., 2019; Nardiello et al., 2019). Metals such as mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), cobalt (Co) and zinc (Zn) are known to have toxic effects on flora and fauna components that are important for the sustainability of the ecosystem (Shahbaz et al., 2013; Raza et al., 2017; Celik et al., 2021).

It is important to select representative indicator species for the continuity of ecosystem health (Mansouri et al., 2012). Some species analyzed to measure the levels of metal contaminants are important bioindicator tools (Stankovic et al., 2014). Birds, which are among the important components of wildlife, are useful bioindicators for determining environmental metal pollution (Mukhtar et al., 2020; Karakaş, 2023). The high mobility of this living group, which feeds at different trophic levels, increases its exposure to pollutants (Zamani-Ahmadmahmoodi et al., 2010; Abdennadher et al., 2011). The exposure of birds to pollutants is an increasingly growing concern, as more than 13% of current bird species are threatened globally, and 1.9% are threatened by anthropogenic pollution, particularly metal contamination (IUCN, 2024).

Biomonitoring is defined as the examination of bio-species or ecological communities that respond in observable ways to any disturbance in the natural environment for the purpose of assessment. In comparison with laboratory toxicological investigations, biomonitoring studies offer a more comprehensive perspective on the impact of anthropogenic disturbances and pollutants on the health of specific species and biological communities. Birds have been demonstrated to be highly sensitive to environmental changes (Jones et al., 2023), and they are widely used as indicators of habitat quality, environmental pollution, biodiversity decline and potential future disease outbreaks in humans (Izah et al., 2023).

Birds absorb heavy metals from water, food and other habitats and accumulating them at high concentrations in their tissues and organs. It is also known that birds remove heavy metals from their bodies by storing them in their tissues, eggs, egg shells, feces and feathers (Battaglia et al., 2005; Durmuş et al., 2018). Since there is no animal ethics problem for samples collected from road kills, which has increased recently, the potential for bioindicator monitoring has increased (Azizoğlu and Adızel, 2018; Lin et al., 2019)). These deaths show how serious the ecological impacts of highways are. Ecotoxicological factors, especially heavy metal pollution, negatively affect both the health of living organisms and the balance of the ecosystem. Birds killed along highways can be an tool for monitoring of environment.

Bird feathers belonging to different taxa have been used in many ecotoxicological studies (Abdullah et al., 2015; Yamac et al., 2019; Kushwaha, 2016; Zarrintab et al., 2016). For this reason, the following factors play an important role in sampling and analyzing feathers: i) they accumulate some metals (As) in greater amounts than other body organs do; ii) they play an important role in the excretion of heavy metals from the body (Hg, As, etc.); iii) they are easy to sample (live, dead or museum samples); and iv) they provide the opportunity to study endangered species (Celik et al., 2021).

Therefore, in our study, feather samples of carcasses from the Hakkari-Van highway were used for ecotoxicological monitoring.

The aim of our study was to compare the levels of heavy metal (loid), such as As, Cu, Pb, Ni, Cr, Cd, and Zn, that accumulate in the feathers of carcasses of bird species detected on roads. It also aims to reveal how species are affected by environmental pollution and the differences in metal(loid) levels between species. In order to achieve this objective, it was to take samples of the feathers and detect their heavy metal content using an inductively coupled plasma optical emission spectrometer.

## 2 Materials and methods

### 2.1 Study area

The study was carried out on highways within the Hakkari–Van provincial borders. The total length of the highway is 295 km, including single and double lanes. The project area highway starts from Van city center and passes through the Gürpınar, Başkale, and Hakkari-Center and Çukurca districts. The region under scrutiny is characterized by geographical heterogeneity, encompassing mountainous regions with wooded habitats, as well as steppe areas marked by rock hills and streams. Potential sources of environmental pollution include agricultural regions and mining operations in the immediate vicinity (Figure 1).

### 2.2 Collection of feather samples

The collection of samples was conducted over the period from 1 April 2021 and 30 May 2022. The majority of carcasses were detected during the spring (April-June) and autumn (September-November) bird migration periods.

In the present study, the carcasses of 19 individuals from 11 species across three families (Accipitridae, Falconidae and Strigidae) were examined. These belonged to two raptor orders (Accipitriformes and Strigiformes). The birds were found deceased following observations and scans carried out along the road during routine fieldwork on Van-Hakkari Road.

A composite sample of primaries, secondaries and coverts was collected by plucking from a variety of predatory birds. The collection included 19 specimens of the *Accipiter nisus*-Eurasian Sparrowhawk (n=5), *Buteo buteo*-Eurasian Buzzard (n=1, Buteo rufinus-Long-legged Buzzard (n=2), *Pernis apivorus*-European

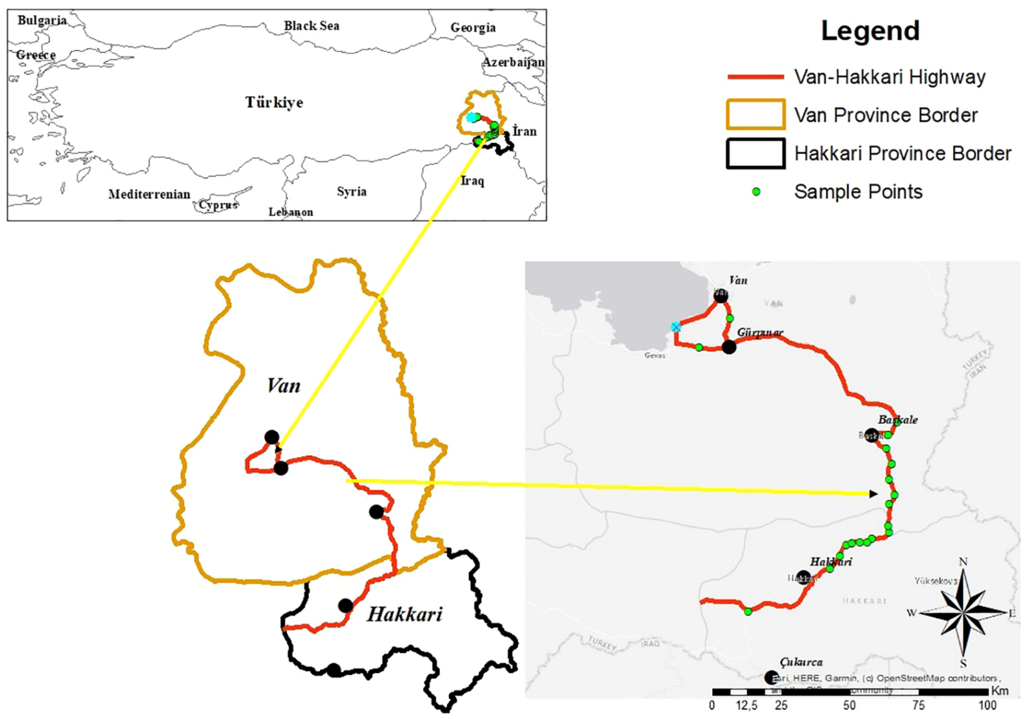


FIGURE 1
Van-Hakkari highway location map.

Honey-buzzard (n=1), *Circaetus gallicus*-Short-toed Snake-eagle (n=1), *Aquila chrysaetos*-Golden Eagle (n=1), *Circus macrourus*-Pallid Harrier (n=1), Falco tinnunculus-Common Kestrel (n=2), *Falco subbuteo*-Eurasian Hobby ((n=2) and *Asio otus*-Northern Long-eared Owl (n=2). However, due to the challenges in sampling raptors, the number of samples obtained was limited. This limitation arises from the rarity of these species in the wild (Mateo et al., 2003). The carcasses were examined and identified, placed in labeled plastic bags and kept in ice coolers for safe and sound preservation (Shahbaz et al., 2013). It was then brought to the Hakkari University Biodiversity Application and Research Center Laboratory and stored in deep freezers for use in subsequent studies. The feathers collected from the carcasses were subsequently separated according to their type (primary wing feathers, secondary wing feathers and breast feathers), kept in sterile, zip-lock bags and stored in deep freezers at -20°C until analysis (Shahbaz et al., 2013; Orłowski et al., 2010, 2019) (Table 1).

## 2.3 Preparation of materials for analysis

The feathers were washed vigorously with alternating deionized water and acetone and then allowed to dry to remove the external contaminants loosely adherent to them (Burger et al., 2009; Frantz et al., 2012; Grúz et al., 2019). Then, the samples were kept in an oven at 60–80°C for 24 hours (Hargreaves et al., 2010; Shahbaz et al., 2013; Yamac et al., 2019; Nergiz and Şamat, 2019).

The dried feather samples were meticulously divided into minute fragments using stainless steel scissors (Seoane et al., 2018). The dry weight of the feathers cut into small pieces was weighed (Shahbaz et al., 2013). One gram (dry weight) of primary and secondary wing feathers and 0.5 g (dry weight) of breast feathers were taken and placed in glass dissolution containers, and a mixture of nitric acid (65% $HNO_3$) and hydrogen peroxide (30% H2O2) was added and maintained at room temperature (Burger et al., 2009; Hargreaves et al., 2010; Yamac et al., 2019; Nergiz and Şamat, 2019; Grúz et al., 2019; Panda et al., 2020). The samples were subsequently placed on a hot plate and evaporated by gradually increasing the temperature until the smoke color changed from black to white (Nergiz and Şamat, 2019; Tuğyan and Sungur, 2020). The digested samples were filtered with Whatman filter paper, cooled, diluted with deionized water, placed in falcon tubes, and stored at -20°C until analysis (Ashkoo et al., 2020). The samples were then analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

## 2.4 Statistical analyses

All samples were read on an ICP–OES (Accreditation No: AB-1332-T and Blank 0,5-1,3,5,7) device at Van Yüzüncü Yıl University Research and Application Center. The results were compared via ANOVA and the Kruskal–Wallis and Hochberg tests in the R statistical software program. Descriptive statistics (means and standard deviations) were used to characterize the trace element loadings. Additionally, normally distributed data were analyzed with one-way ANOVA. Data that were not normally distributed were analyzed via the Kruskal–Wallis test. All the results were

TABLE 1 Species to which the feather samples used in the study belong.

| Ordo | Familia | Species | N | General diet |
|---|---|---|---|---|
| Accipitriformes | Accipitridae | *Accipiter nisus*-Eurasian Sparrowhawk | 5 | |
| | | *Buteo buteo*-Eurasian Buzzard | 1 | Small mammal eating |
| | | *Buteo rufinus*-Long-legged Buzzard | 2 | Small mammal eating |
| | | *Pernis apivorus*-European Honey-buzzard | 1 | Wasps and hornets eating |
| | | *Circaetus gallicus*-Short-toed Snake-eagle | 1 | Snake eating |
| | | *Aquila chrysaetos*-Golden Eagle | 1 | Small mammal eating |
| | | *Circus macrourus*-Pallid Harrier | 1 | Birds eating |
| | Falconidae | *Falco tinnunculus*-Common Kestrel | 2 | Birds eating |
| | | *Falco subbuteo*-Eurasian Hobby | 2 | Birds eating |
| Strigiformes | Strigidae | *Asio otus*-Northern Long-eared Owl | 2 | Small mammal eating |
| | | *Bubo bubo*-Eurasian Eagle-owl | 1 | Small mammal eating |

TABLE 2 Average metal (loid) levels (μg/g) in three regions (primary wing, secondary wing and breast feathers) of families.

| Familia | As* | Cd$^{ns}$ | Cr$^{ns}$ | Cu | Ni$^{ns}$ | Pb$^{ns}$ | Zn* |
|---|---|---|---|---|---|---|---|
| Strigidae | 0,18± | LOD | 0,26± | 0,91± | 0,24± | 0,18± | 13,08± |
| Falconidae | 0,07± | LOD | 0,58± | 1,49± | 0,44± | 0,26± | 20,76± |
| Accipitridae | 0,11± | 0,03± | 0,35± | 0,95± | 0,26± | 0,20± | 18,36± |

*LOD, below the limit of detection. The values change from red to green. Red and green indicate decreases and increases respectively.

considered significant when p<0.05. In our study, characteristics including age, sex, body weight, and biometric measurements were unavailable for all bird species; hence, these variables were not included in our analysis.

# 3 Results

In our study, the amounts of As, Cd, Cr, Cu, Ni, Pb, and Zn in the primary wing feathers, secondary wing feathers, and breast feathers of 19 individuals of 11 species collected from 3 families (Accipitridae, Falconidae, Strigidae) belonging to 2 raptor orders (Accipitriformes, Strigiformes) found dead on the Van-Hakkari highway were measured.

## 3.1 Comparison of metal(loid) levels by families

The average metal(loid) amounts of feathers from three regions (primary wing, secondary wing and breast feathers) taken from 3 samples of 2 species belonging to the Strigidae family of the Strigiformes order and 4 samples of 2 species belonging to the Falconidae family of the Accipitriformes order were measured as Zn>Cu>Cr>Ni>Pb>As. Cd values could not be measured in the Strigidae and Falconidae families because they remained substandard. The average metal(loid) amount of the 11 samples from the 7 species belonging to the Accipitridae family were as follows: Zn>Cu>Cr>Ni>Pb>As>Cd (Table 2).

When the metal(loid) levels of primary wing feathers were compared according to their families, the average values of the Strigidae (0.0904 μg/g) family were determined to be Zn>Cu>Ni>Cr>Pb>As (Table 3). The Cd concentrations in the primary wing feathers of the Strigidae family were below the standard.

In the family Falconidae (0.1031 μg/g), the average metal(loid) levels in primary wing feathers are Zn>Cu>Cr>Ni>Pb>As (Table 3). The Cd values remained below the standard in all individuals. In addition, only one individual of the species Falco subbuteo had As, Ni, Pb and Zn values below the standard.

In the family Accipitridae (0.1777 μg/g), the average metal(loid) levels in primary wing feathers are in the order of Zn>Cu>Ni>Cr>Pb>Cd>As (Table 3). The Cd levels in the primary wing feathers were below the detection limit in one individual of the eurasian buzzard (*Buteo buteo*), the golden eagle (*Aquila chrysaetos*), and the european honey-buzzard (*Pernis apivorus*) and in two individuals of the long-legged buzzard (*Buteo rufinus*).

According to the analysis results, when the metal(loid) levels in the primary wing feathers of the species were compared, the species with the highest As, Cr, Ni, and Pb values was the pallid harrier (*Circus macrourus*). The species with the highest Cd value was eurasian sparrowhawk (*Accipiter nisus*). The species with the highest Cu value belongs to the kestrel (*Falco tinnunculus*), and the highest Zn value belongs to the long-legged buzzard (*B. rufinus*).

When the average metal(loid) levels of secondary wing feathers were compared according to their families, they were ranked as Zn>Cu>As>Cr>Ni>Pb in the Strigidae family (0,1775 μg/g) (Table 4). Cd values in all species of the Strigidae family and Ni values in the secondary wing feathers of an individual of the long-eared owl (*Asio otus*) were below the standard.

In the Falconidae family (0,0742 μg/g), secondary wing feather metal(loid) levels were measured as Zn>Cu>Cr>Ni>Pb>As (Table 4). The Cd values remained below the standard in all individuals. Additionally, the As value in one individual of the kestrel (*F. tinnunculus*) species was below the standard.

TABLE 3 Metal (loid) levels in primary wing feathers of different species and mean metal (loid) levels at the family level (µg/g).

| Familia | Species | | As | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| | *Bubo bubo* | | 0.0649 | LOD | 0.27 | 1.40 | 0.48 | 0.21 | 13.94 |
| | *Asio otus* | | 0.098 | LOD | 0.20 | 0.96 | 0.11 | 0.17 | 9.83 |
| | *Asio otus* | | 0.1083 | LOD | 0.48 | 1.77 | 0.50 | 0.28 | 22.55 |
| | | Mean | 0.0904 | | 0.32 | 1.38 | 0.36 | 0.22 | 15.44 |
| Strigidae | | SD | 0.0226 | | 0.15 | 0.40 | 0.22 | 0.05 | 6.49 |
| | *F.tinnunculus* | | 0.0637 | LOD | 1.15 | 2.14 | 0.76 | 0.33 | 22.23 |
| | *F.tinnunculus* | | 0.1329 | LOD | 0.98 | 1.98 | 0.57 | 0.17 | 15.43 |
| | *F. subbuteo* | | 0.1126 | LOD | 0.35 | 1.60 | 0.23 | 0.30 | 19.33 |
| | *F. subbuteo* | | 0 | LOD | 0.09 | 0.04 | LOD | LOD | LOD |
| | | Mean | 0.1031 | | 0.64 | 1.44 | 0.52 | 0.27 | 19.00 |
| Falconidae | | SD | 0.0356 | | 0.51 | 0.96 | 0.27 | 0.08 | 3.41 |
| | *Accipiter nisus* | | 0.14 | 0.0002 | 0.35 | 1.04 | 0.17 | 0.19 | 13.14 |
| | *Accipiter nisus* | | 0.1208 | 0.0043 | 0.31 | 0.80 | 0.08 | 0.12 | 12.25 |
| | *Accipiter nisus* | | 0.2758 | 0.0175 | 0.55 | 2.01 | 0.90 | 0.40 | 28.11 |
| | *Accipiter nisus* | | 0.1445 | 0.0146 | 0.45 | 1.69 | 0.58 | 0.45 | 34.26 |
| | *Accipiter nisus* | | 0.1516 | 0.0017 | 0.34 | 1.34 | 0.05 | 0.29 | 18.40 |
| | *Pernis apivorus* | | 0.1413 | LOD | 0.25 | 0.95 | 0.09 | 0.07 | 9.79 |
| | *Buteo buteo* | | 0.0782 | LOD | 0.27 | 0.75 | 0.18 | 0.24 | 19.09 |
| | *Circaetus gallicus* | | 0.2856 | 0.2252 | 0.19 | 0.42 | 0.68 | 0.51 | 36.70 |
| | *Aquila chrysaetos* | | 0.064 | LOD | 0.25 | 0.51 | 0.11 | 0.11 | 16.47 |
| | *Circus macrourus* | | 0.5571 | 0.038 | 1.08 | 1.88 | 1.78 | 0.53 | 17.81 |
| | *Buteo rufinus* | | 0.06 | LOD | 0.73 | 2.39 | 0.73 | 0.39 | 38.06 |
| | *Buteo rufinus* | | 0.1142 | LOD | 0.84 | 0.94 | 0.34 | 0.33 | 50.94 |
| | | Mean | 0.1777 | 0.0431 | 0.47 | 1.23 | 0.47 | 0.30 | 24.59 |
| Accipitridae | | SD | 0.1394 | 0.0814 | 0.28 | 0.63 | 0.51 | 0.16 | 12.83 |

The values change from red to green. Red and green indicate decreases and increases respectively.

In the Accipitridae family (0,1128 µg/g), the secondary wing feather metal(loid) levels were as follows: Zn>Cu>Cr>Ni>Pb>As>Cd (Table 4). In addition, the Ni values remained substandard in one individual each of the eurasian sparrowhawks (*A. nisus*) and the european honey-buzzard (*P. apivorus*). Cd was measured only in the pallid harrier (*C. macrourus*) individual.

When the metal(loid) levels in the secondary wing feathers of bird species were compared, the highest arsenic (As) level was detected in the eurasian eagle owl (*Bubo bubo*), the highest nickel (Ni) level was detected in the pallid harrier (*C. macrourus*), the highest lead (Pb) level (0.66272 µg/g) was detected in the eurasian sparrowhawk (*A. nisus*), and the highest zinc (Zn) level (43.56134 µg/g) was detected in the long-legged buzzard (*B. rufinus*). The common kestrel (*F. tinnunculus*) had the highest levels of chromium (Cr) and copper (Cu) among the studied species. The cadmium (Cd) level was measured at 0.01485 mg/g only in the pallid harrier (*C. macrourus*), whereas it remained below the standard detection limit in all other species.

When the metal(loid) levels in breast feathers were compared by family, the order in the Strigidae family was determined to be Zn > Cu > Cr > Ni > Pb > As (Table 5). In an individual of the long-eared owl (*A. otus*), the As and Ni values were below the standards. In the Falconidae family, the metal(loid) levels in breast feathers were measured in the order of Zn > Cu > Cr > Ni > Pb > As (Table 5).

In the Accipitridae family, the metal(loid) levels in breast feathers were ranked in the order of Zn > Cu > Cr > Pb > Ni > As. Ni levels in individuals of the golden eagle (*Aquila chrysaetos*), short-toed snake eagle (*Circaetus gallicus*), pallid harrier (*C. macrourus*), and long-legged buzzard (*B. rufinus*) were below the standard detection limits. In addition, As levels were below the standard detection limit in individuals of the honey buzzard (*P. apivorus*) and the eurasian

TABLE 4 Metal (loid) levels in secondary wing feathers of different species and mean metal (loid) levels at the family level (μg/g).

| Familia | Species | | As | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| | *Bubo bubo* | | 0,3692 | LOD | 0,2159 | 0,7769 | 0,1998 | 0,1756 | 11,354 |
| | *Asio otus* | | 0,0552 | LOD | 0,2465 | 1,2686 | LOD | 0,172 | 18,9074 |
| | *Asio otus* | | 0,1082 | LOD | 0,3075 | 1,9461 | 0,0855 | 0,1954 | 23,8371 |
| | | Mean | 0,1775 | | 0,2566 | 1,3305 | 0,1427 | 0,181 | 18,0328 |
| Strigidae | | SD | 0,1681 | | 0,0466 | 0,5871 | 0,0808 | 0,0126 | 6,2873 |
| | *Falco tinnunculus* | | 0,0709 | LOD | 0,4779 | 2,4488 | 0,4063 | 0,2269 | 26,1071 |
| | *Falco tinnunculus* | | LOD | LOD | 1,1822 | 2,3032 | 0,5003 | 0,3122 | 26,2883 |
| | *Falco subbuteo* | | 0,0782 | LOD | 0,3512 | 2,3822 | 0,1562 | 0,4354 | 23,1932 |
| | *Falco subbuteo* | | 0,0733 | LOD | 1,1751 | 1,7012 | 1,0239 | 0,554 | 49,2191 |
| | | Mean | 0,0742 | | 0,7966 | 2,2089 | 0,5217 | 0,3821 | 31,2019 |
| Falconidae | | SD | 0,0037 | | 0,3847 | 0,2976 | 0,316 | 0,1238 | 10,4745 |
| | *Accipiter nisus* | | 0,0545 | LOD | 0,1999 | 0,5356 | 0,0362 | 0,0926 | 8,4045 |
| | *Accipiter nisus* | | 0,2307 | LOD | 0,2077 | 0,7147 | LOD | 0,0484 | 8,4886 |
| | *Accipiter nisus-* | | 0,0239 | LOD | 0,5269 | 1,5677 | 0,5219 | 0,2276 | 24,9309 |
| | *Accipiter nisus* | | 0,1985 | LOD | 0,2547 | 0,8406 | 0,0532 | 0,1558 | 14,5686 |
| | *Accipiter nisus* | | 0,061 | LOD | 0,2417 | 0,8047 | 0,0347 | 0,1384 | 16,2615 |
| | *Pernis apivorus* | | 0,0856 | LOD | 0,1675 | 0,9118 | LOD | 0,0548 | 15,7073 |
| | *Buteo buteo* | | 0,0684 | LOD | 0,2713 | 0,4575 | 0,0027 | 0,1206 | 13,2402 |
| | *Circaetus gallicus* | | 0,0829 | LOD | 0,3049 | 1,7756 | 0,0809 | 0,1823 | 25,8185 |
| | *Aquila chrysaetos* | | 0,141 | LOD | 0,5784 | 0,9324 | 0,2366 | 0,004 | 33,4593 |
| | *Circus macrourus* | | 0,1195 | 0,0149 | 0,6025 | 1,2113 | 0,6008 | 0,3737 | 20,5846 |
| | *Buteo rufinus* | | 0,1591 | LOD | 0,8303 | 1,7466 | 0,5731 | 0,3691 | 53,6258 |
| | *Buteo rufinus* | | 0,1288 | LOD | 0,5684 | 0,9284 | 0,2338 | 0,2044 | 33,4968 |
| | | Mean | 0,1128 | | 0,3962 | 1,0356 | 0,2374 | 0,1643 | 22,3822 |
| Accipitridae | | SD | 0,0618 | | 0,2143 | 0,4455 | 0,2406 | 0,117 | 13 |

The values change from red to green. Red and green indicate decreases and increases respectively.

sparrowhawk (*A. nisus*). The Cd levels were below the standard detection limit in the breast feathers of all the species.

When metal(loid) levels in breast feathers were compared, the highest As level was measured in the euasian eagle owl (*B. bubo*), the highest Zn level was measured in the long-eared owl (*A. otus*), and the highest Cr and Ni levels were measured in the kestrel (*F. tinnunculus*). Additionally, the short-toed snake eagle (*C. gallicus*) had the highest Cu and Pb levels (Table 5). The Cd levels were below the standard detection limit in all the samples.

## 3.2 Comparison of metal(loid) levels according to feather type

When primary wing feathers, secondary wing feathers, and breast feathers were compared, the order of As, Cr, Ni and Pb amounts was as follows: primary wing feathers>secondary wing feathers>breast feathers. The order of Cu and Zn amount was secondary wing feathers>primary wing feathers>breast feathers. Since the Cd values were below the standard detection limit in all the breast feather samples and in all the secondary wing feather samples except for one (C. *macrourus*), comparisons could not be made (Table 6).

## 3.3 Statistical analysis

For the statistical analyses, the families were compared via ANOVA, the Kruskal–Wallis test, and the Hochberg test via the R program. Since the number of samples taken from each species differed, the Hochberg test was chosen as the *post hoc* method. When p values were examined with the Hochberg test, all of them were $p>0.05$, and there was no difference between the groups. This result shows that the n values are very small. According to Supplementary Table S1, there is a significant difference in the average Cu metal

TABLE 5 Metal (loid) levels in the breast feathers of the species and mean metal (loid) levels at the family level (µg/g).

| Familia | Species | | As | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| | *Bubo bubo* | | 0,0789 | LOD | 0,1872 | 0,5065 | 0,1553 | 0,1321 | 14,2934 |
| | *Asio otus* | | 0 | LOD | 0,2173 | 0,4443 | LOD | 0,1247 | 8,9982 |
| | *Asio otus* | | 0,0543 | LOD | 0,3039 | 0,7977 | 0,1675 | 0,1679 | 14,0341 |
| | | Mean | 0,0666 | | 0,2362 | 0,5828 | 0,1614 | 0,1415 | 12,4419 |
| Strigidae | | SD | 0,0174 | | 0,0606 | 0,1886 | 0,0086 | 0,0231 | 2,9852 |
| | *Falco tinnunculus* | | 0,0243 | LOD | 0,203 | 0,7768 | 0,106 | 0,0806 | 10,1972 |
| | *Falco tinnunculus* | | 0,0416 | LOD | 0,4146 | 1,2302 | 0,2373 | 0,1168 | 14,6409 |
| | *Falco subbuteo* | | 0,0563 | LOD | 0,1545 | 0,6322 | 0,0617 | 0,1105 | 5,7749 |
| | *Falco subbuteo* | | 0,0201 | LOD | 0,4034 | 0,6917 | 0,6798 | 0,1568 | 17,7702 |
| | | Mean | 0,0355 | | 0,2939 | 0,8327 | 0,2712 | 0,1162 | 12,0958 |
| Falconidae | | SD | 0,0167 | | 0,1345 | 0,2716 | 0,2824 | 0,0313 | 5,2356 |
| | *Accipiter nisus* | | 0,0467 | LOD | 0,129 | 0,4304 | 0,0085 | 0,0718 | 8,7662 |
| | *Accipiter nisus* | | LOD | LOD | 0,2452 | 0,3281 | 0,0779 | 0,1177 | 4,3282 |
| | *Accipiter nisus* | | 0,1168 | LOD | 0,2112 | 0,4387 | 0,2659 | 0,2464 | 6,6815 |
| | *Accipiter nisus* | | 0,0392 | LOD | 0,1775 | 0,702 | 0,0186 | 0,1385 | 9,9697 |
| | *Accipiter nisus* | | 0,0486 | LOD | 0,1428 | 0,353 | 0,0949 | 0,0672 | 5,1585 |
| | *Pernis apivorus* | | 0 | LOD | 0,0945 | 0,6003 | 0,0229 | 0,03 | 4,5296 |
| | *Buteo buteo* | | 0,0378 | LOD | 0,1958 | 0,4864 | 0,0681 | 0,1384 | 6,9816 |
| | *Circaetus gallicus* | | 0,0729 | LOD | 0,2523 | 2,2932 | LOD | 0,3402 | 10,8148 |
| | *Aquila chrysaetos* | | 0,0041 | LOD | 0,1734 | 0,3044 | LOD | 0,051 | 5,8211 |
| | *Circus macrourus* | | 0,0286 | LOD | 0,0715 | 0,2565 | LOD | 0,1345 | 6,0765 |
| | *Buteo rufinus* | | 0,0665 | LOD | 0,2876 | 0,6162 | 0,0679 | 0,1966 | 18,0543 |
| | *Buteo rufinus* | | 0,0252 | LOD | 0,3092 | 0,2206 | LOD | 0,0435 | 10,3219 |
| | | Mean | 0,0486 | | 0,1908 | 0,5858 | 0,0781 | 0,1313 | 8,1253 |
| Accipitridae | | SD | 0,0311 | | 0,0743 | 0,5582 | 0,0821 | 0,0922 | 3,8554 |

*SD, Standard deviation; Mean, Average; LOD, below the limit of detection. The values change from red to green. Red and green indicate decreases and increases respectively.

levels between the Falconidae and Accipitridae families ($p<0.05$). Since Cr and Pb did not have a normal distribution, the Kruskal–Wallis test was used to examine whether the group means of the metals were different according to the family variable. According to the results, there was no statistically significant difference between the groups ($p>0.05$) (Supplementary Table S2).

Since there were not enough data on Cd levels, the data were not processed. As Cr, Ni, and Pb did not provide the assumption of

TABLE 6 Average metal (loid) levels according to feather type (µg/g).

| Feather types | As | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|
| Primary Wing Feather | n:18 | n:7 | n:19 | n:19 | n:18 | n:18 | n:18 |
| | 0,15074 | 0,04307 | 0,48028 | 1,29505 | 0,46302 | 0,28370 | 22,12923 |
| Secondary Wing Feather | n:18 | n:1 | n:19 | n:19 | n:16 | n:19 | n:19 |
| | 0,11717 | 0,01485 | 0,45845 | 1,32914 | 0,29662 | 0,21279 | 23,55225 |
| Cover Feather | n:16 | | n:19 | n:19 | n:14 | n:19 | n:19 |
| | 0,04760 | LOD | 0,21968 | 0,63732 | 0,14517 | 0,12973 | 9,64277 |

*n, number of samples; LOD, below the limit of detection.

homogeneity of variance ($p<0.05$), the Kruskal–Wallis test was used in the analysis of these metal(loid)s (Supplementary Table S3). When As, Ni, Cr and Pb metal (loid)s were evaluated via the Kruskal–Wallis test, no difference was observed between the species groups. ANOVA was used for Zn and Cu metal(loid)s, as it provided the assumption of homogeneity of variance (Supplementary Table S4). There was a difference in Zn metal levels between the groups ($p<0.05$). Since only As met the assumption of homogeneity of variance, ANOVA (Supplementary Table S5) was performed for this metal(loid), and the Kruskal–Wallis (Supplementary Table S6) test was performed for Ni, Cr, Pb and Zn. There was a difference between the groups for As ($p<0.05$). In addition, the results of the Kruskal–Wallis test revealed that there was a difference between the group means for Ni, Cr, Pb, Cu, and Zn ($p<0.05$).

When the general statistical comparative analysis results are examined, it is seen that the mean As, Cr, Ni, Pb, and Zn metal(loid) levels of the Strigidae and Accipitridae families and the mean Cu and Zn metal(loid) levels of the Falconidae and Accipitridae families significantly differed from each other.

# 4 Discussion and conclusion

Environmental issues are of major concern to researchers and have been investigated in depth in recent years, as evidenced by the large number of documents in the SCOPUS database (n=1307).

As indicators, birds play crucial or vital roles in monitoring and quantifying hazardous metals (Stankovic et al., 2014). The samples were analyzed for toxic elements in their different feathers. We hypothesized that the status of feathers, such as primary, secondary, and tertiary feathers, might exhibit different accumulation behaviors since the exposure time and intensity of the metals are not the same.

Raptors are considered to be at a high trophic level. A considerable number of raptors are known to exhibit territorial and non-migratory characteristics, as evidenced by their possession of extensive home ranges and notably long-life spans. Consequently, there is an elevated risk of metals accumulating in these tissues, including soft body tissues, bones, feathers, and eggs (Zaccaroni et al., 2003; Shafaeipour et al., 2024) It is hypothesised that raptor species are more vulnerable to metals (Stout and Trust, 2002; Nighat et al., 2013; Farahani et al., 2015; Kitowski et al., 2016; Zarrintab and Mirzaei, 2018; Elbagory et al., 2025) and are important as bio-indicators (Wayland and Bollinger, 1999; Zaccaroni et al., 2003).

In our study, the amounts of As, Cd, Cr, Cu, Ni, Pb, and Zn in the primary, secondary flight feathers, and breast feathers of 19 carcasses belonging to 11 species collected from 3 families (Accipitridae, Falconidae, and Strigidae) of two raptor orders (Accipitriformes and Strigiformes) on the Hakkari–Van highway were measured for ecotoxicological monitoring. In our analyses, the metal(loid)s with the highest accumulation rates in feathers were Zn>Cu>Cr>Ni>Pb>As>Cd. Additionally, in this study, metal concentrations in the feathers of the pallid harrier (*C. macrourus*) were measured for the first time.

Studies on the feathers of raptor species are relatively rare. As asserted by Burger and Gochfeld (2000), the maximum permissible level of cadmium in avian populations' feathers was determined as 2.00 mg/kg. Cd has been demonstrated to exert a detrimental effect on avian subjects, manifesting in impaired egg formation, testicular damage, oviduct dysfunction, and renal deterioration (Grúz et al., 2019). In a study conducted in Spain, the concentrations of cadmium, mercury and lead were determined in the feathers of northern goshawk (*Accipiter gentilis*), eurasian buzzard (*B. buteo*) and tawny owl (*Strix aluco*). No significant differences in metal concentrations were observed according to age or sex. Cadmium levels in feathers were found to be significantly correlated in all species ($p<0.01$ or $p<0.05$), and in eurasian buzzard (*B. buteo*), a correlation was also observed between cadmium ($p<0.05$) and lead ($p<0.01$) concentrations in feathers (Castro et al., 2011). In our study, Cd levels were below the standard detection limit in the Strigidae and Falconidae families and therefore were not measured. It is posited herein that the avian subjects sampled in the present study may experience mild adverse effects due to environmental cadmium load, even in areas which do not exhibit elevated levels of pollution (Burger and Gochfeld, 2000; Scheuhammer et al., 2007).

The average Pb concentration was 0.17994 µg/g in the Strigidae family, 0.25575 µg/g in the Falconidae family, and 0.199 µg/g in the Accipitridae family. Since Pb did not have a normal distribution, the Kruskal–Wallis test was used to examine whether the group means of the metals differed according to the family variable. The results revealed that there was no significant difference between the groups ($p>0.05$). In a study in northern Spain, heavy metal concentrations were measured in samples of primary, secondary wing and breast feathers taken from tawny owl (*S. aluco*) and northern goshawk (*A. gentilis*) species. High individual variability was observed in all feather types (Debén et al., 2012).

It is evident that both Zn and Cu are essential elements and important compounds in enzymes, bone, immunity, and feather formation (Picone et al., 2019). However, at high levels, they can cause harmful effects, such as problems with the respiratory, reproductive, liver, hormone, and digestive systems (Abdullah et al., 2015).

In a study conducted in the Punjab region of Pakistan, no differences in heavy metal concentrations were observed among three raptor bird families. As concentrations ranging from 1.06 mg/kg to 6.44 mg/kg were measured in four species from the Strigidae family.It was stated that the highest accumulation was measured in the Falconidae family and kestrel (*F. tinnunculus*) species (Nighat et al., 2013). In studies conducted in Norway and Spain, metal concentrations were determined in feather samples from the offspring of the northern goshawk (*A. gentilis*) (Dolan et al., 2017). It was emphasized that the mean concentrations in feathers were 82.0 ± 12.4 and 0.0018 ± 0.002 for Zn and Cd, respectively (Nighat et al., 2013). In another study conducted in northwestern Spain, As, Cd and Pb concentrations were determined in the primary wing feathers of 10 individuals of the tawny owl (*S. aluco*) species (Seoane et al., 2018). It was concluded that As and Cd concentrations were higher in the parts of the feathers closer to the body. In our study, bird species were analyzed according to three regions: primary wing feathers, secondary wing feathers, and breast feathers. When the metal(loid) levels of the primary flight feathers were compared, the average values were as follows: Zn>Cu>Ni>Cr>Pb>As. The metal(loid) levels of the secondary flight feathers were as follows: Zn>Cu>Cr>Ni>Pb>As>Cd. When the breast feathers were compared, the metal(loid) levels were as follows: Zn>Cu>Cr>Ni>Pb>As. Contrary to existing studies, As and Cd were low in all regions in our study.

Nighat et al. (2013), in their study, used the Kruskal–Wallis test, a non-parametric method for comparing multiple independent groups, to demonstrate significant differences in Cd, Zn, Ni, and Pb levels among three avian families ($p < 0.05$). This test is particularly suitable for non-normally distributed data, which is common in environmental toxicology studies (Corder and Foreman, 2014). A significant difference was found between the Accipitridae (e.g., eagles and hawks) and Falconidae (falcons) families for Cd ($p < 0.01$), Zn ($p < 0.01$), Ni ($p < 0.01$), and Pb ($p < 0.05$); the Mann–Whitney U *post-hoc* test results are highlighted. Similarly, the families Falconidae and Strigidae (owls) also presented significant differences in terms of Cd ($p < 0.01$), Zn ($p < 0.01$), Ni ($p < 0.05$), and Pb ($p < 0.01$), as determined by the Mann–Whitney U test. However, the concentrations of all the metals were not significantly different between the Accipitridae and Strigidae families ($p > 0.05$). This suggests that dietary habits, trophic levels, and habitat preferences may play a crucial role in metal bioaccumulation patterns among these bird families (Burger, 1993).

It has been stated that the Falconidae family accumulated the highest metal concentrations, followed by the Accipitridae family and then the Strigidae family. This hierarchy may reflect differences in metabolic rates, foraging behavior, or exposure to contaminated prey, as falcons are apex predators with high metabolic demands (Espín et al., 2016). In our study, families were statistically analyzed in terms of metal(loid) accumulation levels. A significant difference was found between the Falconidae and Accipitridae families in terms of the mean Cu metal concentrations ($p < 0.05$). Copper is an essential element but can become toxic at elevated levels, potentially affecting avian health and reproduction (Scheuhammer, 1987).

According to the Cr and Pb results, there was no significant difference between the groups ($p > 0.05$). This could indicate similar exposure pathways or detoxification mechanisms for these metals across the studied families (Dauwe et al., 2005). When As, Ni, Cr, and Pb were evaluated via the Kruskal–Wallis test, no differences were observed between the species groups ($p < 0.05$). Additionally, it was observed that there was a difference between families for Ni, Cr, Pb, Cu, and Zn ($p < 0.05$). Such variations may be attributed to species-specific physiological processes, including metal sequestration in feathers or excretion efficiency (Jaspers et al., 2019).

There were significant variances ($p < 0.05$) between the mean concentrations of As, Cr, Ni, Pb, and Zn in the Strigidae and Accipitridae families. Owls (Strigidae) usually have lower metal levels than diurnal raptors (Eens et al., 1999) because they feed at night and are less exposed to industrial pollutants.There was significant variation between the mean amounts of Cu and Zn in the Falconidae and Accipitridae families ($p < 0.05$).Zinc is especially important for the functions of enzymes and the production of keratin in feathers. An imbalance in zinc can also mess up immune responses (Nam and Lee, 2006). The results are fairly consistent with other studies that have used feathers as biomonitors, which show that feathers are good indicators of metal exposure in the environment (Movalli et al., 2017). Zinc is a heavy metal that is important for the normal growth of feathers and for the body to work properly.It also protects against the kidney damage that cadmium (Cd) can cause (Malik and Zeb, 2009; Movalli et al., 2018). This is because Zn can compete with Cd for binding sites in metallothionein proteins, which lowers the oxidative stress caused by Cd (Thévenod and Lee, 2013).

Copper is necessary for the structure and function of many proteins that are vital for normal growth, the metabolism of living cells, and cell function (Harms and Buresh, 1987; Pesti and Bakalli, 1996; Underwood and Suttle, 1999). However, high concentrations of Zn and Cu in the body can harm the health of organisms. The highest concentration measured in falcons in Pakistan was 113 mg/kg (Movalli, 2000). In our study, the highest zinc concentration (53.625 µg/gr) was measured in the long-legged buzzard (*B. rufinus*). The permissible Cu limits in various environmental matrices are as follows: 1.3 mg/L in drinking water (USEPA, 2010), 0.1 $mg/m^3$ in smoke, and 1 $mg/m^3$ in fog and dust (Occupational Safety and Health Administration, 2002; Klaassen and Amdur, 2013). In birds, the toxic effects of copper metal have been observed at concentrations ranging from 250 to 1000 mg Cu/kg (Jackson et al., 1979; Jackson and Stevenson, 1981; Chiou et al., 1999). In another study done in Pakistan, the amount of copper found was about 17.64 ± 4.05 mg/kg in Accipitridae, 32.17 ± 13.66 mg/kg in Falconidae, and 7.78 ± 2.48 mg/kg in Strigidae species (Nighat et al., 2013). In our study, the average copper concentrations were 0.949 µg/g in Accipitridae, 1.495 µg/g in Falconidae, and 0.912 µg/g in Strigidae species. In this case, the levels detected in our study were below the specified threshold values and lower than those reported in other studies. Individual instances where toxic metal concentrations increased may have been due to the bird's diet and not external contamination of the feathers (Bhargava et al., 2012; Pandey and Madhuri, 2014; Dai et al., 2016; Giromini et al., 2016; Grúz et al., 2019). Therefore, the low levels observed in our study are likely due to regional factors and bird dietary conditions.

Arsenic (As) is a metalloid with both metal and non-metal properties, but it is often referred to as a metal (Sánchez-Virosta et al., 2015; Celik et al., 2021). Arsenic is a teratogen and carcinogen that can cross placental barriers and cause death and deformities in some species (Eisler, 1998). It often causes toxicity by accumulating in the tissues of animals at the top of the food chain (e.g., *Tyto alba*, *Buteo buteo*, *Accipiter nisus*, *Falco tinnunculus*) (Eisler, 1998). In a study conducted in Hungary, As concentrations were 0.40 ± 0.30 µg/g in the feathers of owl species, 0.33 ± 0.17 µg/g in *Buteo buteo*, 0.29 ± 0.24 µg/g in *Falco tinnunculus*, and 0.32 ± 0.18 µg/g in *Accipiter nisus* (Pesti and Bakalli, 1996). In our study, we found the average As levels to be: 0.36 µg/g in *Bubo bubo*, 0.1 ± 0.05 µg/g in *Asio otus*, 0.07 µg/g in *Falco tinnunculus*, 0.02 ± 0.23 µg/g in *Accipiter nisus*, and 0.06 µg/g in *Buteo buteo*. The results of the above study are largely similar to the As amounts in owl and hawk species, but compared with kestrel and eurasian common buzzard species, the As amount in our study is low.

Malvandi and Shamabadi (2022) examined the concentrations of heavy metals in species such as the common buzzard (*B. buteo*), long-legged buzzard (*B. rufinus*), kestrel (*F. tinnunculus*), and long-eared owl (*A. otus*), which died due to vehicle collisions on highways in Iran. They reported that there was no significant difference in metal concentrations between adult and juvenile individuals, between diurnal and nocturnal raptors, or between species with different diets. In our study, we examined the metal(loid) concentrations of 19 samples of 11 species from three families belonging to two orders found dead on highways in different regions. Accordingly, differences were observed between families and species. Nutritional ecology is a multidisciplinary approach that focuses primarily on living organisms, the environment, and the nutritional foundation of cooperation between organisms (function, mechanism, development) and the environment (biotic and abiotic) (Raubenheimer et al., 2009). Considering the diversity of heavy metals in the environment, avoiding the presence of heavy metals in the food chain and the environment is impossible. These differences in species are assumed to be due to nutritional differences between species.

Bird feathers can be successfully used as indicators of environmental pollution because of the relatively constant ratio of pollutants in the body to the levels found in the feathers, as well as the high correlation between pollutant levels in the feathers (Shehzad et al., 2022). However, feathers are complex and are not easy to collect. Since feather samples can be stored for long periods without deterioration, identifiable bird carcasses are often used to eliminate complexity in feather studies. In our study, the use of feather samples from bird carcasses of different taxa and the presentation of metal concentrations in the feathers of the long-legged buzzard (*C. macrourus*) for the first time will provide a foundation for future research.

In conclusion, our study suggests that raptor feathers could be useful bioindicators for monitoring metal(loid) contamination in eastern Anatolia.The most common element that was examined was zinc (Zn). This observation fits with its role as a necessary micronutrient and a possible ecotoxic agent at high levels.The differences in zinc and other metal(loid) levels between families and feather types are caused by differences in their environment, food, and ecology. This is the first study to find out what amount of metal(loid) is in the feathers of the pallid harrier (*Circus macrourus*). This information will help with future studies in ecotoxicology and conservation. Even though the current levels of zinc aren't dangerous, it's important to keep an eye on them, especially in places where people can hurt the environment. Feather analysis is still a non-invasive, ethical, and broad way to keep an eye on the health of animals in the wild.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The animal study was approved by Ethics Committee of Van Yüzüncü Yıl University. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

ÖÖ: Conceptualization, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Writing – original draft. EA: Conceptualization, Software, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Supervision, Project administration, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2025.1647589/full#supplementary-material

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