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Factors influencing lead, mercury and other trace element exposure in birds from metal mining areas

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ABSTRACT

Non-ferrous metal mining is considered one of the largest sources of toxic metal released to the environment and may threaten ecosystems, notably biota. We explored how birds that inhabit non-ferrous metal mining sites are exposed to mercury, lead, and other trace elements by analyzing their feathers and verifying which factors may influence element concentrations in feathers. We sampled a total of 168 birds, representing 26 species, with different feeding habits and migration patterns in a non-polluted reference site and two historical metal mining areas: Almadén, which is considered one of the most heavily mercury-contaminated sites worldwide, and the Sierra Madrona mountains where lead has been mined since ancient times. The quantification of aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), thorium (Th), thallium (Tl), uranium (U), vanadium (V) and zinc (Zn) was performed by inductively coupled plasma mass spectrometry (ICP-MS). Feather analysis revealed contamination by Hg and Pb, in Almadén and Sierra Madrona, respectively. We found that granivorous birds had the lowest feather Hg levels compared to those found in omnivorous, insectivorous, and piscivorous species, whereas feather Pb was about twice as high in granivores and omnivores, than in insectivorous and piscivorous birds. We also found differences among study sites in 13 elements and confirmed the influence of feather age, migratory patterns of the birds, and external deposition of elements, on metal concentrations in the feathers. Our results highlight that despite the cessation of metal mining in the study areas, local avifauna are being exposed to Hg and Pb from abandoned mines and old tailings sites, indicating that appropriate measures are needed to protect biota from overexposure to these toxic metals.

1. Introduction

Mining and metallurgy, especially of non-ferrous metals, are among the primary sources of emissions of metals and metalloids to the environment. Elements released to the atmosphere, water, and soil due to the extraction, refining, and processing of metal ores may be bioaccessible for living organisms (Rybicka, 1996; Venkateswarlu et al., 2016). Many areas of the world have been affected by the metallurgical industry, which has caused multi-element pollution to local environments (Candeias et al., 2019). Though emitted several centuries ago, metals and metalloids are still available to wildlife living in abandoned metallurgical areas (Camizuli et al., 2018; Rodríguez-Estival et al., 2019). In our paper we have focused on mercury (Hg) and lead (Pb) – two metals that are hazardous for all living organisms, including birds. Mercury is considered a potent neurotoxic element (Driscoll et al., 2013) and can cause numerous adverse health effects in birds (Whitney and Cristol, 2017). Lead is a persistent inorganic pollutant that may affect all body systems, producing hematological, neurological, immunological,

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reproductive, and behavioral disorders in exposed birds (McClelland et al., 2019; Vallverdú-Coll et al., 2019; Williams et al., 2017). These two metals, once released to the environment from mining and smelting, can be taken up by plants and incorporated into their tissues (Kabata--Pendias and Mukherjee, 2007), thus becoming accessible to animals feeding on them, including herbivores, insects, earthworms, and mollusks (Álvarez et al., 2018; Eeva et al., 2018; Laskowski and Hopkin, 1996). The lower trophic level consumers incorporate metals and metalloids into their tissues and serve as food for other animals higher in the trophic chain (Beaubien et al., 2020; Fritsch et al., 2012). Birds are considered valuable bioindicators or biomonitors of environmental pollution because they usually occupy higher levels of the food chain, accumulate different contaminants, and are sensitive enough to reflect environmental changes (Becker, 2003; Furness and Greenwood, 1996). Birds are exposed to metals mostly through dietary and inhalation routes (Sánchez-Virosta et al., 2015; Sanderfoot and Holloway, 2017; Williams et al., 2017). Another potential source of metals and metalloids for birds is geophagy observed among many species (Beyer et al., 1999; Downs et al., 2019; Hui, 2004). The uptake and bioaccumulation of metals and metalloids depend on their bioavailability, route of exposure, and the bird's intrinsic properties, including its trophic position, feeding habits, age, and genetic variability (Burger et al., 2008). Different tissues and matrices are used to assess the exposure of birds to environmental contaminants. Internal tissue analysis is considered the "gold standard" because it gives precise information on long-term exposure to metals and metalloids. The main disadvantage of tissue sampling is that most samples are only available from dead animals, often with an unknown cause of death and at various stages of decay. Blood analysis is another possibility that may provide useful information about the current exposure to contaminants, but blood sampling may be challenging, risky, especially in small passerines (Sánchez-Virosta et al., 2020) and reflects mainly recent exposure rather than body burden from long term exposure. Finally, feather analysis is considered an alternative, less invasive tool for assessing the exposure of birds to certain trace elements, especially Hg (Bottini et al., 2021; Gil-Jiménez et al., 2020). However, it should be noted that element levels in feathers reflect exposure time during which the feathers form and grow (Bottini et al., 2021; Burger, 1993). A complicating factor may be external contamination with dust or soil, showing a spectrum of elements adhering to their surface. This may be considered a disadvantage for assessing bioaccumulation in birds, but can be advantageous as passive biomonitoring reflecting the overall level of pollution of the bird's habitat (Jaspers et al., 2019).

The mining districts of Valle de Alcudia-Sierra Madrona in Central Spain offer the possibility to study different scenarios of metal exposure (i.e., Hg and Pb mines) within the same biogeographical region within 2500 km^2 (Palero-Fernández and Martín-Izard, 2005). Almadén mining district has been the most important producer of Hg for several centuries, providing this metal for gold mining in South America during Spanish colonization (Higueras et al., 2013). Currently, bioaccumulation of mercury is observed through different levels of aquatic food webs, including bivalves (Berzas Nevado et al., 2003), crustaceans (Rodríguez-Estival et al., 2019), fish, turtles (Ortiz-Santaliestra et al., 2019), and river otters (Rodríguez-Estival et al., 2020). Elevated Hg levels have also been reported in terrestrial plants, rodents (Hildebrand et al., 1980) and wild ungulates (Patiño Ropero et al., 2016). However, the information about Hg bioaccumulation in other bird species living in this area is limited (Hildebrand et al., 1980). Moreover, around 500 abandoned Pb mines and test sites are distributed along the Valle de Alcudia-Sierra Madrona district, which produced widespread contamination of soils, sediments, plants (Higueras et al., 2017; Reglero et al., 2008), and animals (Ortiz-Santaliestra et al., 2019; Pareja-Carrera et al., 2014; Reglero et al., 2009; Rodríguez-Estival et al., 2014). Here we present the first study results that show elemental pollution in birds from the Valle de Alcudia-Sierra Madrona district. Bird diversity in the study areas allowed us to verify the effect of different ecological niches on element bioaccumulation.

The main objectives of our study were: 1) to analyze the levels of Hg, Pb and other chemical elements (Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Th, Tl, U, V, Zn) in the feathers of birds representing different ecological traits and inhabiting habitats affected by metal mining; 2) to investigate the relationship between the levels of elements and stable isotopes in bird feathers; 3) to determine the influence of external contamination of feathers; and 4) to assess the extent of exposure of birds inhabiting Hg and Pb mining areas by comparing metal levels in feathers with published concentrations of these elements linked with adverse health effects.

2. Material and methods

2.1. Study sites

The research was performed in two historic mining areas and one control area located in the center of Ciudad Real province in Spain (Fig. 1). The first mining area, Almadén, is considered one of the largest natural deposits of Hg on Earth (Saupe, 1990). The exploitation of Hg ores in the Almadén district started in the Roman Empire and continued until 2003. At that time, about 260,000 t of Hg was produced (Higueras et al., 2011). The natural presence of Hg deposits in the rocks, and the mining activity related to its extraction, contributed to enormous Hg pollution of terrestrial and aquatic ecosystems in the district. The levels of Hg found in the soil of old metallurgical sites of Almadén could reach 8889 μg g^{-1} (Higueras et al., 2003), stream waters 6–11,200 μg L^{-1} and sediments 0.5–16,000 μg g^{-1} (Gray et al., 2004; Higueras et al., 2006). The estimated mean atmospheric Hg concentration in the district in the 20th century was 600 ng m^{-3} (Tejero et al., 2015). Almadén district is a semiarid area with a mean rainfall of 527 mm m^{-2} and an average annual temperature of 15.3 °C. *Dehesa* is a predominant landscape in the district, which means the traditional Spanish land-use system in rural areas, mainly rangelands which are occupied by scattered oak trees (*Quercus rotundifolia, Q. suber,* and *Q. faginea.*) and primarily used for extensive livestock production and hunting (Joffre et al., 1988). The sampling site in Almadén was located on the banks of the Guadalmez River downstream from the mercury mines, approximately 13 km southwest from the town of Almadén (38°43′03.1″N 4°57′30.5″W) at an altitude of about 350 m above sea level (a. s. l.).

The second mining area, Sierra Madrona, is in the eastern part of the Alcudia Valley, the major producer of Pb and other metals in Spain over the past centuries. The mining of Pb–Zn ores in Alcudia Valley has been carried out since the Roman Empire. Lead was produced chiefly by small mines, but there were also several larger producers such as San Quintín, El Horacajo, and Diógenes with production volumes of approximately 500,000, 300,000, and 200,000 t of Pb, respectively (Palero-Fernández and Martín-Izard, 2005). The exploitation of Pb–Zn ores resulted in contamination of soil and biota with Pb and other potentially harmful elements (Higueras et al., 2017; Reglero et al., 2009). The site where birds were captured was close to the Montoro River dam and approximately 1.5 km east from the Diógenes mine (38°31′22.8″N 4°06′00.8″W) at an altitude of about 525 m a. s. l. The Montoro River above and below the dam receives water and sediment from Diógenes mine and the surrounding mountain slopes. The area is characterized by a sub-humid Mediterranean climate with distinct seasons and is covered by *dehesa* landscape (Higueras et al., 2017).

The reference area consisted of two sampling sites located northwest of the city of Ciudad Real (Fig. 1). The first reference sampling point was on the riverbank of the Bullaque River, 7 km southwest from the town of Porzuna (39°06′18.4″N 4°12′47.7″W) at an altitude of approximately 568 m a. s. l. The second one was next to the town of Picón (39°03′45.7″N 4°03′50.0″W) at an altitude of 624 m a. s. l. The reference area shares the same vegetation type, climatic and geographical conditions as Almadén and Sierra Madrona sites, but concentrations of Pb and Hg in the soil, water, and plants in this area are considered background

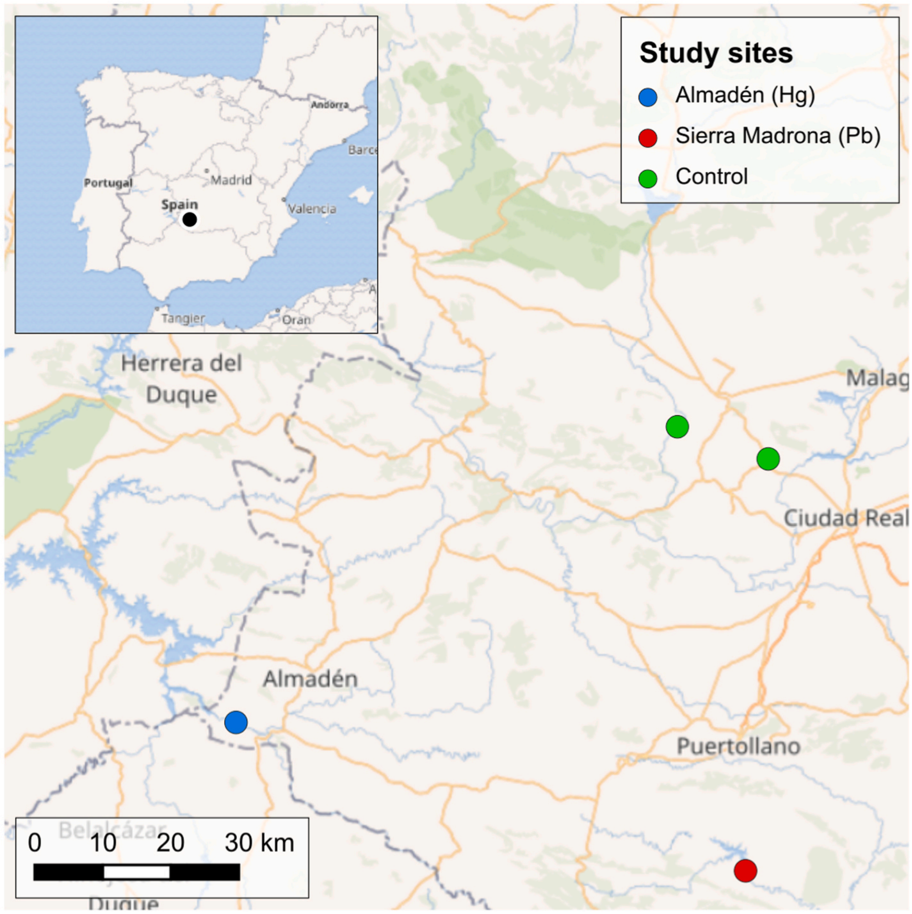

Fig. 1. Location of study sites on the geographical map of Spain.

(Ortiz-Santaliestra et al., 2019; Reglero et al., 2008; Rodríguez-Estival et al., 2020).

2.2. Sampling

A total of 168 birds were mist netted between 8 and 23 of June 2017. Captured birds represented 26 species, including: barn swallow (*Hirundo rustica*), Cetti's warbler (*Cettia cetti*), common chaffinch (*Fringilla coelebs*), common kingfisher (*Alcedo atthis*), common nightingale (*Luscinia megarhynchos*), corn bunting (*Emberiza calandra*), crested lark (*Galerida cristata*), Eurasian blackbird (*Turdus merula*), Eurasian blue tit (*Cyanistes caeruleus*), Eurasian collared-dove (*Streptopelia decaocto*), Eurasian golden oriole (*Oriolus oriolus*), Eurasian great tit (*Parus major*), Eurasian hoopoe (*Upupa epops*), Eurasian jay (*Garrulus glandarius*), Eurasian reed-warbler (*Acrocephalus scirpaceus*), European goldfinch (*Carduelis carduelis*), European greenfinch (*Chloris chloris*), European stonechat (*Saxicola rubicola*), hawfinch (*Coccothraustes coccothraustes*), house sparrow (*Passer domesticus*), Iberian azure-winged magpie (*Cyanopica cooki*), Sardinian warbler (*Curruca melanocephala*), Spanish sparrow (*Passer hispaniolensis*), spotless starling (*Sturnus unicolor*), eastern subalpine warbler (*Curruca cantillans*), and woodchat shrike (*Lanius senator*). Detailed information about the ecological traits of the species and the number of birds captured at each study site are summarized in Supplementary Table S1. The mist nets were placed in wooded areas or near the riverbank, and their exact placings were chosen based on the structure of vegetation and observations of birds at each study site. Erected nets were checked every five to 10 min. Captured birds were extracted carefully from the mist net and kept in cotton holding bags until processing. All necessary information was recorded during the handling of captured birds. Fully developed feathers (secondaries, tertiaries, and tail feathers) lost by birds during capture and handling were collected. In birds with active molt, old feathers close to being lost in the molt sequence were plucked. Then birds were ringed and released into the wild. Each feather was classified as old or new based on the wear of the feather, the molt status and the bird's age (Svensson, 1992). Samples were placed into labeled polypropylene zip-bags and stored at −20 °C until further processing.

2.3. Elemental analysis

For elemental analysis, all feathers taken from the bird were used except one tail feather, which was kept to analyze stable isotopes. Sampled feathers were rinsed vigorously with tap water, homogenized using stainless-steel scissors, placed into polypropylene conical tubes, and sonicated in analytical grade isopropyl alcohol (Panreac Química S. L.U., Barcelona, Spain) for 10 min, followed by sonication in 1% nitric acid (HNO_3) solution for 10 min, sonicated in Milli-Q™ water for the next 10 min and then rinsed thoroughly with Milli-Q™ water and finally dried in open tubes at 50 °C overnight. Approximately 100 mg of pooled and homogenized feather sample from one individual was weighed accurately into an acid-washed quartz digestion tube. A volume of 2 ml of 67% nitric acid (HNO_3) (NORMATOM®, VWR International, Leuven, Belgium) was added to each sample. Then, samples were heated for 1 h at the temperature of 100 °C in a heating block (JP Selecta, Barcelona, Spain) and then cooled to the ambient temperature. Subsequently, a volume of 0.5 ml of non-stabilized 30% H_2O_2 (Suprapur®, Merck, Darmstadt, Germany) was added to each digestion tube, and another two-step heating process was performed (1 h at 90 °C and 2 h at 120 °C). For each digestion batch, one reagent blank and one sample of certified reference material (CRM) were processed. After digestion, samples were cooled to the ambient temperature, transferred into acid-washed polypropylene tubes, made up to the final volume of 10 ml with Milli-Q™ water, and stored at ambient temperature until analysis.

The elemental analyses were carried out at the laboratory of the Department of Pharmacology and Toxicology of the National Veterinary Research Institute in Puławy, Poland (NVRI) as described previously by Durkalec et al. (2018) with a slight modification. Briefly, the concentrations of trace elements were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using a 7700× spectrometer (Agilent Technologies, Tokyo, Japan). The quantification of Be, Mg, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Ba, Tl, Pb, Th, and U was based on a five-point external calibration curve (from 0.5 to 250 μg L^{-1}) prepared by dilution of multi-element standard stock solution (10 μg/ml) IV-ICPMS-71A (Inorganic Ventures, Christiansburg, VA, USA). The analysis of Hg was performed separately based on a three-point calibration curve (from 0.1 to 5 μg L^{-1}) prepared from Hg standard stock solution (10 μg/ml) MSHG-10PPM (Inorganic Ventures, Christiansburg, VA, USA). The multi-element standard solution containing 200 μg L^{-1} of Bi, Li, Ho, In, Rh, Sc, Tb, and Y was prepared by diluting 6020 ISS stock solution (Inorganic Ventures, Christiansburg, VA, USA) and used as the online internal standard. A tuning solution for ICP-MS 7500cs containing 1 μg L^{-1} of Ce, Co, Li, Mg, Tl, and Y (Agilent Technologies, Santa Clara, CA, USA) was used to carry out daily optimization of the mass analyzer. All operating conditions are included in Supplementary Table S2. The trueness of the calibration curve was monitored using the Trace Elements in Water certified reference material SRM-1643f (NIST, Gaithersburg, MD, USA). The quality of measurements was verified by analysis of trace elements in the following CRMs: SRM-1577c Bovine Liver (NIST, Gaithersburg, MD, USA), BCR-185R Bovine liver (IRMM, Geel, Belgium), ERM-BB186 Pig kidney (IRMM, Geel, Belgium), and TORT-3 Lobster hepatopancreas (NRC, Ottawa, Canada). The recoveries were summarized in Supplementary Tables S3-S5. The results are expressed in μg g^{-1} of dry weight (d. w.).

2.4. Stable isotope analysis

For the analysis of stable C and N isotopes, samples of feathers were prepared separately to remove the external contamination according to the procedure provided by the laboratory. Briefly, one tail feather was placed in a 15 ml polypropylene conical tube and sonicated twice in analytical grade ethanol (Panreac Química S.L.U., Barcelona, Spain) for 10 min with subsequent throughout rinsing with deionized water after each sonication. Next, feather samples were sonicated with deionized water for 10 min, rinsed thoroughly with Milli-Q™ water, and dried in open tubes at 50 °C overnight. Then, feathers were homogenized using stainless steel scissors and forceps, weighed to the nearest 1 mg into tin capsules, and sent to the laboratory for the isotopic analysis. The analysis of δ^{13}C and δ15N was performed at the Stable Isotope Laboratory of Doñana Biological Station (LIE-EBD, Sevilla, Spain; http://www.ebd.csic.es/lie/Home.html) using a continuous flow isotope-ratio mass spectrometry system by Flash HT Plus elemental analyzer coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany) as described by Ramírez et al. (2015). The method allows determining precisely δ^{13}C and δ^{15}N with acceptable measurement errors of ±0.1‰ and ±0.2‰, respectively. Stable isotope ratios are expressed in the standard δ-notation (‰) relative to Vienna Pee Dee Belemnite (δ^{13}C) and atmospheric N_2 (δ^{15}N).

2.5. Data treatment and statistical analysis

All analyses were performed using R version 4.0.2 (R Core Team, 2020). The *dplyr* package version 1.0.2 was used for data manipulation and calculation of the descriptive statistics (Wickham et al., 2020), the *ggplot2* version 3.3.1 (Wickham, 2016), and the *ggpubr* version 0.3.0 (Kassambara, 2020) packages were used for result visualization. The number of sampled birds was not sufficiently representative for each species to test the effect of taxonomy on the specific element concentrations in bird feathers. We consider this a major limitation of our study (Supplementary Table S1). We wanted to test whether selected ecological niches of birds could predict the content of elements in their feathers. Therefore, we categorized all studied species of birds into four feeding habit categories: granivorous (N = 78), omnivorous (N = 24), insectivorous (N = 50), and piscivorous birds (N = 16) based on information on their main food items. Bird species were also categorized by migration patterns into two categories: migratory (N = 18) and resident (N = 150). The information about main food items and movement patterns of particular species was adapted from the Birds of the World web page of the Cornell Lab of Ornithology (Billerman et al., 2020). We are aware that the bird molting pattern may vary between species and age groups, and some of the migratory species studied may have grown their feathers on wintering grounds far from our study areas. Thus, we have added additional information about the molting pattern of the individual bird (SC - complete molt in the summer or WC - complete molt during winter) (Svensson, 1992), which indicates whether an individual grew its feathers in the study area. In our study, most of the samples (97%) were from birds that grew their feathers in the study area, so this variable was excluded from our analysis. Left-censored data (below the LOQ) were set as half of the LOQ. The normality of data distribution was verified using the Shapiro-Wilk test (Yap and Sim, 2011). Since the distribution of trace element and stable isotope data was not-normal, we used log-transformation to achieve the normality. Generalized linear models (GLMs) were used to verify the influence of the following factors on the concentration of each element in the feathers: ratio of stable nitrogen isotopes (δ^{15}N), ratio of stable carbon isotopes (δ^{13}C), study site (Almadén, Control, and Sierra Madrona), feeding habit (granivorous, omnivorous, insectivorous, and piscivorous), age of feathers (new or old), and movement pattern (migratory or resident). Since Al is known as a metal with low oral bioavailability, its presence in feathers must be of exogenous origin and could be used to indicate external contamination (Cardiel et al., 2011). Thus, we decided to add the concentration of Al as an explanatory variable to verify the source of the particular element in feathers. The GLM models with Gaussian distribution were constructed as follows: we used log-transformed concentration of the studied element as a dependent variable and all factors including the concentration of Al, δ^{15}N, δ^{13}C, feeding habits, age of feathers, and migration pattern as explanatory variables. The *step* command with a forward-backward stepwise procedure based on Akaïke's Information Criterion (AIC) was used to choose the best fitting model. Wald Chi-square test implemented in *mdscore* package version 0.1–3 (da Silva-Júnior et al., 2014) was used to check if explanatory variables in the model were significant. The differences in element concentrations between the particular factors were verified on log-transformed values by the Tukey HSD or Student's t-test as post-hoc tests using the *emmeans* package version 1.4.7 (Lenth, 2020). A Principal Component Analysis (PCA) was also performed to reduce the number of dependent variables to three main principal components (PC1, PC2, and PC3) that were tested as dependent variables in the GLM with the site, feeding habit, age of feathers, and migration pattern groups as explanatory variables. The PCA was computed with element concentrations by *FactoMineR* package version 1.42 (Lê et al., 2008) and visualized by *factoextra* package version 1.0.7 (Kassambara and Mundt, 2020). The relationships between elements and between elements and ratios of stable isotopes were verified using Spearman's rank correlation coefficient and visualized by *corrplot* package version 0.84 (Wei and Simko, 2017).

3. Results and discussion

Concentrations of trace elements and the results of the stable isotope analyses are summarized in Supplementary Tables S6 and S7. The GLM analysis was performed to investigate which variables influenced the concentrations of each of the studied elements in the feathers. The best-fitting GLM models are summarized in Table 1. These models were then used to calculate the marginal mean concentrations of elements for the categorical explanatory variables. We also performed a PCA analysis with element concentrations to reduce the number of dependent variables. The obtained PC scores were subsequently used as dependent variables in the GLM analysis as with the individual elements (Table 1). The PCA of element concentrations revealed three principal components that explained 61.9% of the variance (Supplementary Fig. S1A). The first principal component (PC1, 46.2% of the explained variance) was influenced by 13 elements (Al, Fe, Ni, V, Th, Cr, Ba, As, Be, U, Co, Mn, and Mg) (Supplementary Fig. S1B). The PC2 (11.5% of explained variance) was influenced by Zn, Se, δ^{15}N, Cu, Mg, and Mo (Supplementary Fig. S1C) and the PC3 (7.9% of explained variance) by Tl, Cd, Cu, Pb, U, Be, Mg, Hg. and δ^{15}N (Supplementary Fig. S1D).

Table 1
The best generalized linear models (GLMs) describe concentrations of trace elements in feathers and principal component values. The models were chosen based on Akaïke information criterion (AIC). Predictors in bold font are statistically significant within the model ($p < 0.05$, Wald's Chi-square test). Arrows symbolize differences between categories of the predictor versus the reference, or the relationship between the element of concern and the predictor.

Dependent variable	Explanatory variables
Al	**Feeding habits** (I↓***, O↓***, P↓***) + **Migration** (R↑**) + Age of feathers (↓^{NS}) + δ^{15}N (↑^{NS})
As	**Al** (↑***) + **Site** (C↓***, SM↓***) + **δ^{15}N** (↑**) + Migration (R↑^{NS})
Ba	**Al** (↑***) + **δ^{15}N** (↑***) + **Feeding habits** (I↑***, O↑^{NS}, P↓^{NS}) + **Migration** (R↑**)
Be	**Al** (↑^{p}***) + **Site** (C↓*, SM↓***) + **δ^{13}C** (↓**) + **δ^{15}N** (↑*)
Cd	**Age of feathers** (N↓***) + **Al** (↑**) + **Migration** (R↓*)
Co	**Al** (↑***) + **Age of feathers** (N↓***) + **δ^{15}N** (↑***) + **Feeding habits** (I↓ **, O↓^{NS}, P↓**) + **δ^{13}C** (↓*)
Cr	**Al** (↑***) + **Age of feathers** (N↑*) + **Feeding habits** (I↓^{NS}, O↑^{NS}, P↓**) + **δ^{15}N** (↑**) + δ^{13}C (↓^{NS})
Cu	**Feeding habits** (I↓*, O↑^{p}***, P↓^{NS}) + **δ^{15}N** (↑***) + **Al** (↑***) + **δ^{13}C** (↓***) + Age of feathers (N↑^{NS}) + **Site** (C↓*, SM↓^{NS})
Fe	**Al** (↑***) + **Feeding habits** (I↓***, O↓^{NS}, P↓***) + **Site** (C↓***, SM↓^{NS}) + **δ^{15}N** (↑**) + Migration (R↑^{NS})
Hg	**Site** (C↓***, SM↓***) + **Feeding habits** (I↑***, O↑**, P↑***)
Mg	**Al** (↑***) + **δ^{15}N** (↑***) + Site (C↓^{NS}, SM↑^{NS}) + δ^{13}C (↓^{NS})
Mn	**Al** (↑***) + **δ^{15}N** (↑***) + **Age of feathers** (N↓***) + **Site** (C↓^{NS}, SM↑^{NS}) + **Feeding habits** (I↓^{NS}, O↑^{NS}, P↑**) + Migration (R↑^{NS})
Mo	**Feeding habits** (I↑***, O↓***, P↑^{NS}) + **Age of feathers** (N↑***) + **δ^{15}N** (↑^{p}**) + **Al** (↑*) + Migration (R↑^{NS}) + δ^{13}C (↓^{NS})
Ni	**Al** (↑***) + **Site** (C↓**, SM↑^{NS}) + **Feeding habits** (I↓*, O↓^{NS}, P↓*) + **Age of feathers** (N↓*) + Migration (R↑^{NS})
Pb	**Al** (↑***) + **Site** (C↓***, SM↑**) + **Feeding habits** (I↓***, O↑^{NS}, P↓***) + **δ^{15}N** (↑**) + Age of feathers (N↓^{NS})
Se	**δ^{15}N** (↑***) + **Site** (C↓***, SM↓***) + **Feeding habits** (I↑*, O↑***, P↑***) + **δ^{13}C** (↓***) + **Al** (↑*) + Age(N↓^{NS}) + Migration (R↓^{NS})
Th	**Al** (↑***) + **Feeding habits** (I↓*, O↑^{NS}, P↓***) + **δ^{15}N** (↑**) + **Site** (C↑^{NS}, SM↓*) + **δ^{13}C** (↓*)
Tl	**Al** (↑***) + **Site** (C↓***, SM↓^{NS}) + **δ^{13}C** (↓*) + Age of feathers (N↑^{NS})
U	**Al** (↑***) + **Site** (C↓*, SM↓*) + **Feeding habits** (I↑^{NS}, O↑*, P↓^{NS}) + Migration (R↑^{NS}) + Age of feathers (N↓^{NS})
V	**Al** (↑***) + **Site** (C↓*, SM↓*) + **Feeding habits** (I↓^{NS}, O↑^{NS}, P↓***) + **δ^{15}N** (↑*) + δ^{13}C (↓^{NS})
Zn	**δ^{15}N** (↑***) + **Feeding habits** (I↑**, O↑***, P↑**) + **Al** (↑**) + δ^{13}C (↓^{NS})
PC1	**Feeding habits** (I↓***, O↓***, P↓***) + **Age of feathers** (N↓***) + **Migration** (R↑*)
PC2	**Feeding habits** (I↑***, O↑***, P↑***) + Migration (R↑^{NS}) + **Site** (C↓*, SM↑^{NS})
PC3	**Site** (C↓^{NS}, SM↓***) + **Feeding habits** (I↑*, O↓*, P↑^{NS})

I – Insectivores, O - Omnivores, P – Piscivores; SM – Sierra Madrona, C – Control; N – new feathers; R - resident; ^{NS} – not significant; * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$.

3.1. External contamination

Metals and metalloids could come from different sources in feathers. Some elements may be accumulated in feather keratin during feather formation and growth, or may adhere to the external surface of feathers due to wet and dry deposition, contact with contaminated soil (e.g., when dustbathing or foraging), immersion in contaminated water, and through the excretion of the uropygial gland during preening (Burger, 1993; Dauwe et al., 2002; Ek et al., 2004; Pilastro et al., 1993; Weyers et al., 1988). External contamination of feathers may complicate interpretation of analytical results in studies on the exposure of birds to particular elements. Normally, cleaning of feathers before elemental analysis is necessary for correct interpretation, but such cleaning never completely removes the contamination adhered or adsorbed onto the feather. On the other hand, external contamination on the feather surface may give information about the presence of pollutants in the bird's habitat (Jaspers et al., 2019). Only a few elements are known to be highly bioaccumulative (Hg and Se) or constitutive in feathers, such as Cu and Zn (Borghesi et al., 2017). Other elements, including As, Pb, Cd, may accumulate in the keratin structure to a lesser extent (Borghesi et al., 2016). Lastly, some elements do not accumulate in feather keratin at all, and their presence in feather samples is caused solely by external contamination of the feather surface. Aluminum is an excellent example of those elements that belong to the last group, as it is known to be of external origin in feathers, and therefore can serve as an indicator of surface contamination of feathers with inorganic particulates (Borghesi et al., 2016; Cardiel et al., 2011). Although various cleaning procedures have been proposed to reduce external contamination before analysis, none of them can completely remove exogenous metallic deposits from feather surfaces (Aloupi et al., 2020). In our study, we used a multistep washing procedure using water, isopropyl alcohol, and diluted nitric acid supported with ultrasound treatment. Despite thorough cleaning before the analysis, high levels of Al were found in samples of feathers (Supplementary Table S6). Because of that, we decided to include Al as a predictor in the GLMs to verify if the concentration of any particular element was influenced by external contamination. The GLM analyses revealed that concentrations of all elements, except Hg, were highly influenced by Al (Table 1). This corroborated findings from Spearman's rank correlation test that confirmed positive correlations between Al and As, Ba, Be, Cd, Co, Cr, Fe, Mg, Mn, Ni, Pb, Th, Tl, U, and V, and negative ones between Al and Hg, Mo, and Se (Fig. 2). Also, the results of the PCA showed strong relationships between the group of elements that were related to PC1, including Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Th, Tl, U, and V (Fig. 3A, Supplementary Fig. S1B). Our results revealed that feather Al was positively associated with concentrations of most of the other trace elements, indicating that their deposition onto feather surfaces may serve as an indirect, but reasonably accurate reflection of birds' total exposure. The one exception was the concentration of Hg in feathers, which was confirmed both by the GLM analysis (Table 1), results of the PCA (Fig. 3A), and negative correlation with Al (Fig. 2). This was consistent with previous research that demonstrated the endogenous origin of Hg in bird feathers (Hahn et al., 1993; Jaspers et al., 2004) and confirmed their usefulness for the assessment of bird's exposure to this toxic element (Bottini et al., 2021).

3.2. Influence of feeding habits

Bioaccumulation of trace elements in avian tissues, eggs and feathers depends largely on the trophic position of a particular bird species, their feeding habits, and type of food consumed (Abbasi et al., 2015; Berglund et al., 2011; Burger, 2002). Many studies have included stable isotope analyses in blood, tissue samples, or epidermal growths of birds to characterize their trophic position and food sources. The ratio of stable nitrogen isotopes (δ^{15}N) is used to describe trophic position, whereas the ratio of stable carbon isotopes (δ^{13}C) provides information on the origin of the carbon source. However, linking the results of Hg and δ^{15}N and

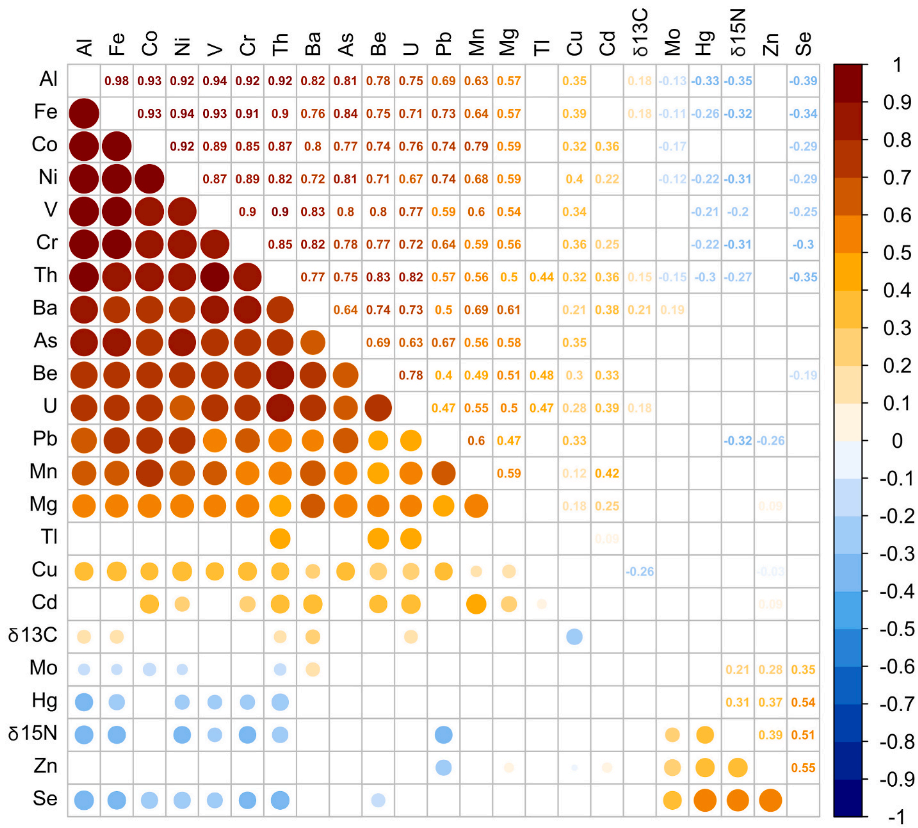

Fig. 2. Spearman rank correlation coefficients (ρ) between trace elements in bird's feathers. The color and size of the circle correspond to the level of correlation: with 1 indicating positive correlation (dark red) and −1 indicating the negative correlation (dark blue). Numerical values of correlations are presented in the lower-left part of the matrix. For clarity, non-significant correlations ($p > 0.05$) are left blank. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

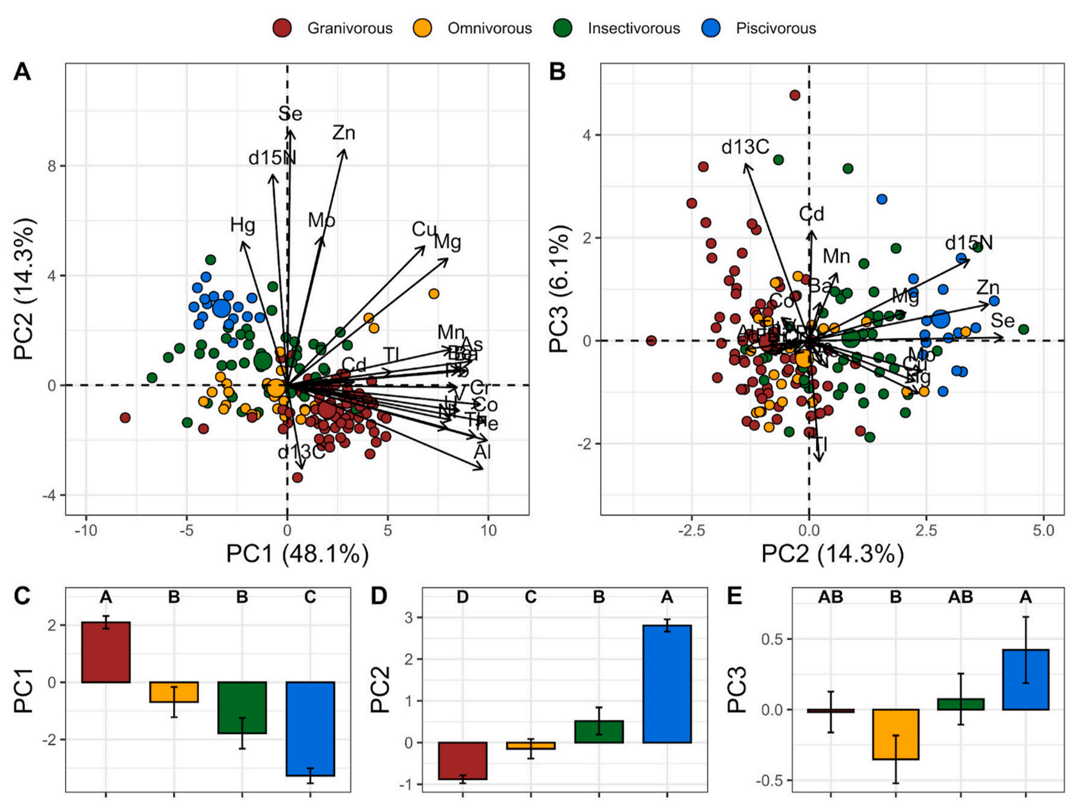

Fig. 3. PCA biplots of trace element loadings (presented as vectors) and the individuals scores of PC1 and PC2 (A); PC2 and PC3 (B) according to the bird feeding habits (color scale). Barplots (C–D) show values of PC scores (mean ± SEM) by the feeding habits of birds. Different letters above bars denote significant differences between groups ($p \leq 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

$\delta^{13}C$ in feathers may not always be possible because of different kinetics in stable $\delta^{15}N$ and $\delta^{13}C$ and toxic elements (Bond, 2010). This complication was evident in our findings because the GLM did not reveal any relationship between Hg and $\delta^{15}N$ (Table 1), and revealed only a weak positive correlation between Hg and $\delta^{15}N$ (Fig. 2). By using $\delta^{15}N$ analysis, we wanted to confirm the general dietary preferences of the bird species studied and their trophic position. Our results confirmed differences in $\delta^{15}N$ between feeding habit groups: granivores = omnivores < insectivores < piscivores (Supplementary Fig. S2). The feeding habits of birds influenced feather concentrations of 16 out of 21 elements tested, particularly Hg and Pb (Table 1). The estimated marginal means of these elements by feeding habit group are summarized in Fig. 4 and Supplementary Table S8. Birds are exposed to Hg primarily as dietary MeHg (Scheuhammer et al., 2007). Mercury bioaccumulates along the food web gradient, and its levels rise with an increase of the trophic level, such that Hg in detritivores < herbivores < omnivores < piscivores (Atwell et al., 1998; Kidd et al., 2012). We found the lowest feather Hg in granivorous birds compared to omnivorous, insectivorous, and piscivorous species. Although the statistical analysis did not confirm differences in feather Hg levels between omnivorous, insectivorous, and piscivorous birds, a visible pattern of increase could be observed along of the trophic level (Fig. 4). We found the highest feather Hg levels in piscivorous birds (Fig. 4). In our study, piscivores were represented by one species – the common kingfisher. Its diet consists mainly of fish (99.93%) but also non-fish prey such as crayfish, frogs, insects, and even small mammals (Čech and Čech, 2015). Results of similar studies on Hg accumulation in birds representing different feeding habits corroborate our findings (Alleva et al., 2006). The authors found more than four-fold higher concentrations of Hg in the liver of piscivorous than in other feeding-habit groups of birds from the Urbino-Pesaro province in Italy. A similar pattern was observed in birds from different provinces in Iran, where mean feather concentration of Hg was four-fold higher compared to herbivorous and insectivorous birds (3.07 μg g^{-1} vs. 0.84 and 0.64 μg g^{-1}, respectively) (Zolfaghari et al., 2009). Our results were also in line with those reported by Ackerman et al. (2019) and Ma et al. (2021), which found higher Hg levels in species of birds that prefer animal-based diet compared to those that prefer mostly plant-based food items.

Our results differ slightly from those of Abeysinghe et al. (2017) in

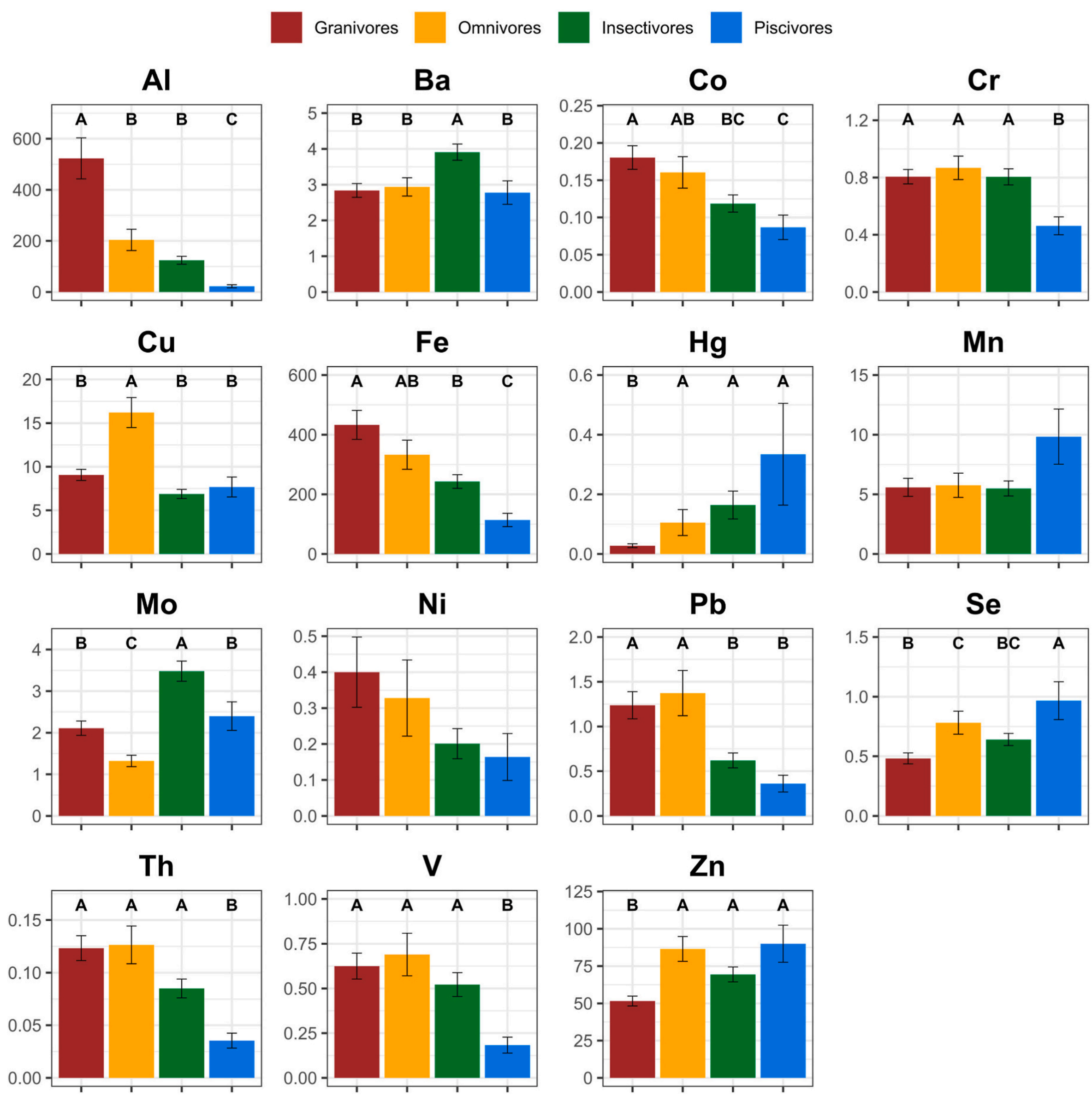

Fig. 4. Estimated marginal means (±SEM) of trace elements in feathers (in μg g−1 of dry wt.) by bird feeding habits calculated based on GLM models with Al as a covariate. The results were averaged based upon the study site, age of feathers, and migration pattern. Different letters above bars denote significant differences between groups ($p \leq 0.05$).

the food web of rice fields cultivated in an inactive Hg mining district in China, where the highest feather Hg levels were found in insectivorous birds than in granivorous and piscivorous species. The authors found similar levels of Hg in the feathers of granivores and kingfisher, which was explained by the high proportion of rice (with high Hg levels) in the diet of granivores (Abeysinghe et al., 2017). Our results were also in contrast with those reported by Leonzio et al. (2009) in passerine birds from Montepulciano wetlands, located in an agricultural area, where feather Hg concentration in insectivorous and granivorous species was more than six times higher than in omnivorous ones. Similarly to Hg, the lowest feather Se and Zn were found in granivores (Fig. 4, Supplementary Table S8).

Lead levels in feathers have been documented as having endogenous and exogenous origins (Dauwe et al., 2002). Feather Pb levels could reflect dietary exposure of birds to this metal, which was confirmed in experimental studies on blue tit nestlings only orally exposed to lead acetate (Markowski et al., 2013). In our study, granivorous and omnivorous birds had concentrations of Pb more than twice higher than insectivorous and piscivorous species (Fig. 4, Supplementary Table S7). Our findings were in line with the results of Chapa-Vargas et al. (2010) from mining areas in southern Mexico, where granivorous, omnivorous, and insectivorous-frugivorous birds had blood Pb levels twice higher than exclusively insectivorous species. The authors hypothesized that ingestion of food items contaminated with dust that contained Pb was the main reason for elevated blood Pb in birds that prefer grains, seeds, herbs, and fruits (Chapa-Vargas et al., 2010). According to Bennett et al. (2011), accidental soil ingestion and intentional grit ingestion are important sources of Pb for birds. Birds that feed on hard plant material need to have a certain amount of grit in their gizzards which aids in grinding larger food particles to make them digestible (Best and Stafford, 2002). Gizzards of granivorous birds may contain more grit than those of insectivorous, omnivorous, and frugivorous ones (Gionfriddo and Best, 1996). Birds that live in areas affected by metal mining have access to mine tailings or other rock waste that likely contain a substantial amount of toxic metals, including Pb. Such material ingested directly or with food items, may contribute to the endogenous origin of Pb in feathers. We believe that feeding behavior, i.e., feeding on the ground, contributes to higher feather Pb in granivores and omnivores

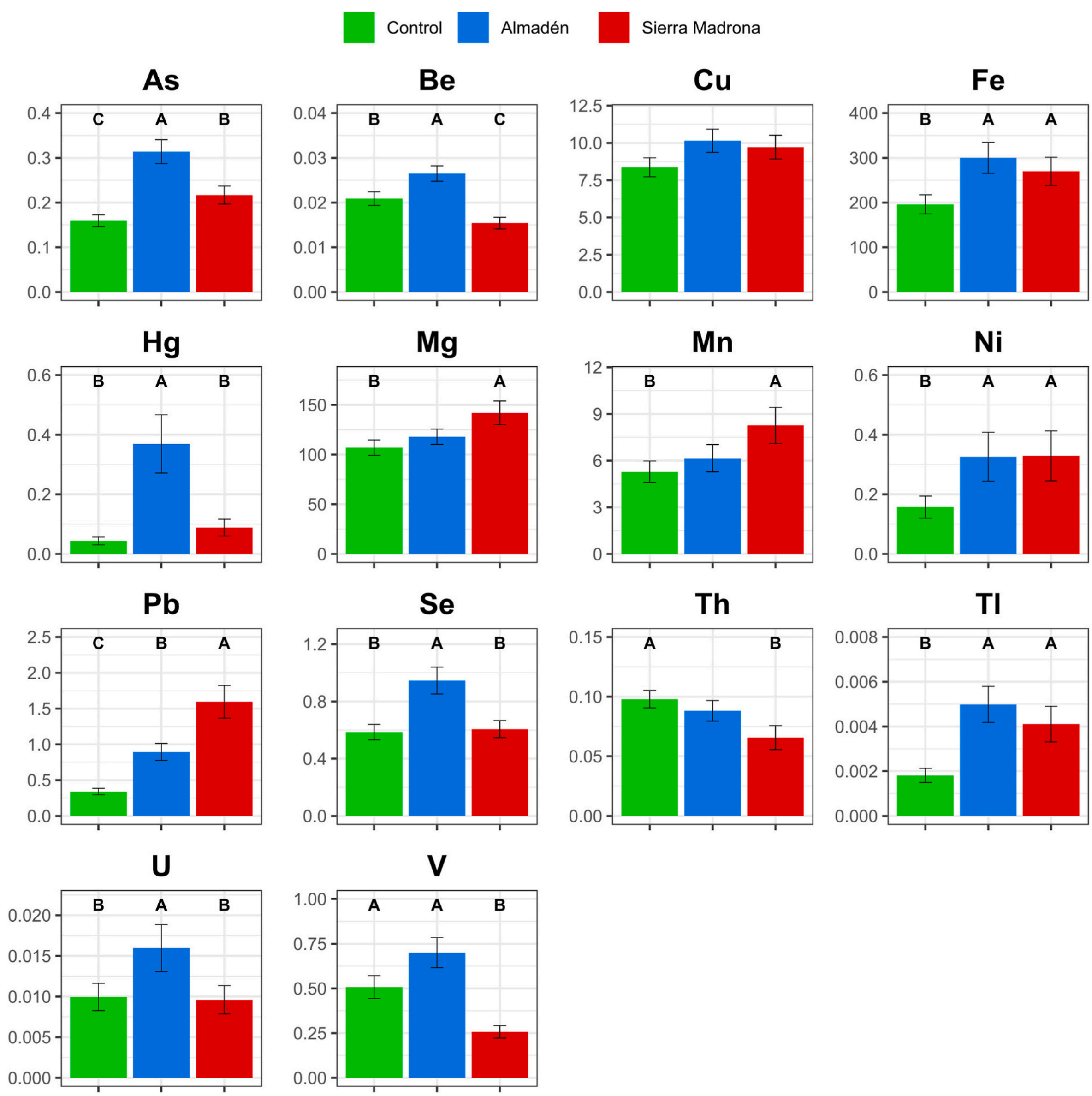

Fig. 5. Estimated marginal means (±SEM) of trace elements in bird feathers (in μg g−1 of dry wt.) by study site calculated based on GLM models with Al as a covariate. The results were averaged over the levels of feeding habits, age of feathers, and migration pattern. Different letters above bars denote significant differences between groups ($p \leq 0.05$).

due to dust exposure. These species may also be exposed to metal-rich particles when dustbathing close to mine waste deposits which contributes to both “internal” and external contamination of feathers with Pb or other related metals such as Al, Co, and Fe. The highest feather Al was found in granivorous birds (523 μg g^{-1}), which was almost twice, four-fold and 23-fold higher than in omnivorous, insectivorous, and piscivorous birds (Fig. 4). A similar pattern was observed for Co, and Fe, where concentrations of those two metals decreased as follows granivores > omnivores > insectivores > piscivores. Mean concentrations of Ni in feathers showed the same pattern as Al, Co, and Fe, but those differences were not confirmed statistically. GLM analysis of PC1, PC2, and PC3 scores confirmed that feeding habits influenced concentrations of elements. PC1 scores were positively impacted by granivorous type of feeding than in omnivorous, insectivorous, and piscivorous (Fig. 3C), whereas PC2 scores showed the opposite pattern (Fig. 3D). Negative PC3 scores were found in omnivorous species compared to other feeding habit groups (Fig. 3E).

3.3. Influence of site

Our results showed that the study site influenced the feather concentrations of 14 elements (As, Be, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Se, Th, Tl, U, and V) (Table 1). Estimated mean concentrations of As, Be, Hg, Se, and U in feathers of birds from Almadén were higher compared to those of birds captured in Sierra Madrona and the reference site ($p \leq 0.05$) (Fig. 5, Supplementary Table S9). The average concentration of Hg in feathers of birds from Almadén (0.369 μg g^{-1}) was more than four- and eight-fold higher than in Sierra Madrona (0.088 μg g^{-1}) and control sites (0.044 μg g^{-1}), respectively ($p \leq 0.05$). Our results of Hg differed from those reported from small terrestrial birds living in areas of Hg mining worldwide. He et al. (2020) analyzed concentrations of Hg in feathers of different bird species from a heavily polluted area in Guangxi province in southern China, where Hg was mined for near 50 years. The authors found feather Hg more than five-fold higher than that reported in our study from the Almadén site. Our results of Hg were also lower than those found in the European bee-eater (*Merops apiaster*) from an abandoned cupric-pyrite mine in southeast Portugal (Lopes et al., 2010), and less than half that reported in different bird species by Sierra-Marquez et al. (2018) from sites contaminated by artisanal gold mining in northwestern Colombia. However, our results were almost eight-fold higher than that found in feathers of song sparrows (*Melospiza melodia fallax*) from an area of Sonoita Creek (Arizona, USA), which receives contaminants from a variety of natural and anthropogenic sources, including runoff from urban areas, active and abandoned mines, as well as effluent from a waste-water treatment plant, after the upgrading of waste-water facilities (Lester and Van Riper, 2014). Regarding Pb, the highest estimated marginal mean concentration was found in feathers of birds from the Sierra Madrona site (1.596 μg g^{-1}), followed by Almadén (0.893 μg g^{-1}), and control (0.340 μg g^{-1}). Our results of feather Pb in birds from Sierra Madrona differ from results reported from other sites affected by metal mining. The average Pb level from Sierra Madrona birds was more than 22-fold lower compared to levels reported in different bird species by He et al. (2020) from the area of Yulan mine located in Guangxi province in China, and approximately half lower than those found in birds from different industrial areas in Pakistan (Abbasi et al., 2015). However, the authors studied only resident bird species, including also carnivorous and scavengers. Feather Pb levels in small terrestrial birds may be influenced by the proximity of the bird’s habitat to the emission source. Janssens et al. (2001) analyzed feathers of great tits (*Parus major*) nesting in areas at different distances from a metal smelter in Belgium, showing that feather Pb was correlated with the distance between tit nests and the source of pollution.

The level of Pb in birds that nested closely to the smelter (<350 m) reached 230.5 μg g^{-1} and decreased by approximately 76.3% and 96.5% in those nesting 2.5 km and 20 km away from the smelter, respectively (Janssens et al., 2001). Although our sampling site at Sierra Madrona was located approximately 1.5 km east of one of the abandoned Diógenes mine, territories of sampled birds may have been larger and not overlap with the area affected by the mine. Similar to Pb, birds from Sierra Madrona had higher feather concentrations of Mg and Mn compared that those captured in control site (142 vs. 107 μg g^{-1} and 8.27 vs 5.28 μg g^{-1}, respectively). Birds from both mining sites had more than twice higher levels of Ni and Tl than those living in control one (Fig. 5 and Supplementary Table S9).

3.4. Influence of feather’s age

The age of feathers can affect the levels of metals and metalloids, which can be of both internal and external origin. Old plumage that has been exposed to the environment for a long period of time is expected to contain higher external load of metals than newly grown feathers (Burger, 1993). In our study, we found that feather concentrations of Al, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Tl and Zn were influenced by the age of feathers (Table 1). Cadmium levels in old feathers were almost three times higher than in new ones (Fig. 6, Supplementary Table S10). A similar difference was found for Mn, where the concentration of this metal in old feathers was twice as high compared to freshly grown feathers. We also noticed significant but slightly lower differences in concentrations of Co and Ni between old and new feathers (Fig. 6). Also, the levels of Al, Pb, Se and U seemed to be higher in old feathers than in new ones, but these differences were not statistically confirmed. The opposite patterns were observed for Cr and Mo, where concentrations of these elements were about 20% higher in new than in old feathers (Fig. 6, Supplementary Table S10).

3.5. Influence of migration

The results of GLM analysis showed that migration status affected the concentration of several elements in the feathers (Al, As, Ba, Cd, Fe, Mn, Mo, Ni, Se, and U) (Table 1). Resident species had Al in their feathers almost twice as high as migratory ones (175.60 μg g^{-1} vs 97.90 μg g^{-1}, $p \leq 0.05$). A similar difference was found in Ba levels between resident and migratory birds (3.59 μg g-1 vs. 2.66 μg g^{-1}, $p \leq 0.05$). The opposite trend was observed for Cd, where this toxic metal level was more than twice as high in migratory birds (0.010 vs. 0.004 μg g^{-1}, $p \leq 0.05$). Selenium levels as well as As, Fe, Mn, Mo, Ni, and U were higher in migratory birds than residents, but the difference was not significant (Fig. 7, Table S11). This was likely a result of unequal sample sizes in both groups of birds, which we addressed as a limitation of this work. Our study showed no effect of migration status on feather Hg and Pb levels. Ackerman et al. (2019) found that blood Hg levels in birds making a summer or winter stopover in the Central Valley of California, or year-round residents had higher Hg levels than in migratory species. However, they found no differences in feather Hg content among the groups of birds studied, which supports our results.

Other authors who studied Hg levels in aquatic birds in the Gulf of California found higher mercury concentrations in residents compared to migratory birds; however, the authors suggest that this was due to dietary differences rather than migration status (Ruelas-Inzunza and Páez-Osuna, 2004). Cooper et al. (2017) studied blood and feather accumulation of different metals in resident northern cardinal (*Cardinalis cardinalis*) and migratory great-crested flycatcher (*Myiarchus crinitus*) to check if migration may determine metal exposure. The flycatchers were exposed to different levels of heavy metals during feather formation on their wintering grounds compared to those they were recently exposed to on their breeding grounds.

3.6. Potential adverse effects of Hg and Pb

Mercury exposure may cause many deleterious effects in birds, such as lowered reproductive success, behavioral and neurological disorders, oxidative stress, altered immune response and hormone levels (Whitney

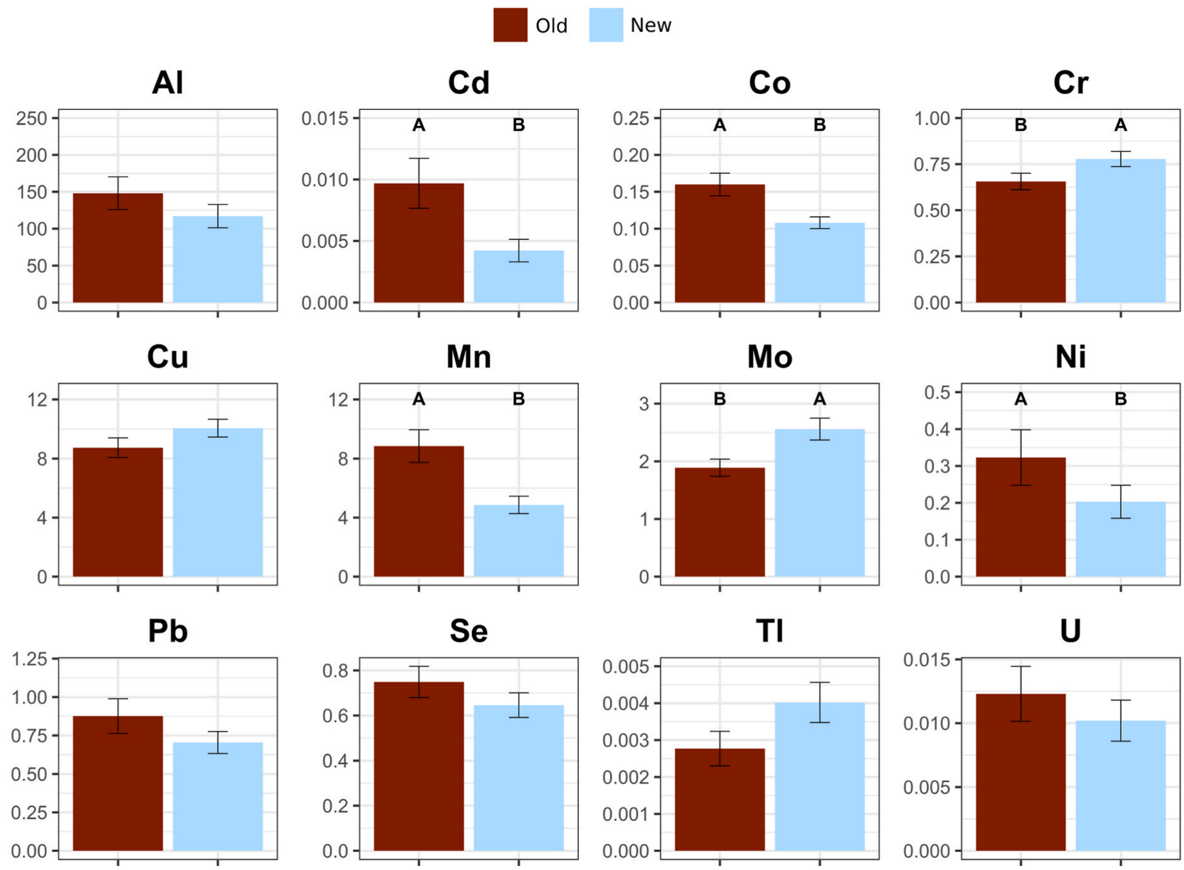

Fig. 6. Estimated marginal means (±SEM) of trace elements in old and new feathers (in μg g−1 of dry wt.) calculated based on GLM models with Al as a covariate. Different letters above bars denote significant differences between groups ($p \leq 0.05$). The results were averaged over the levels of feeding habits, study site, and migration pattern.

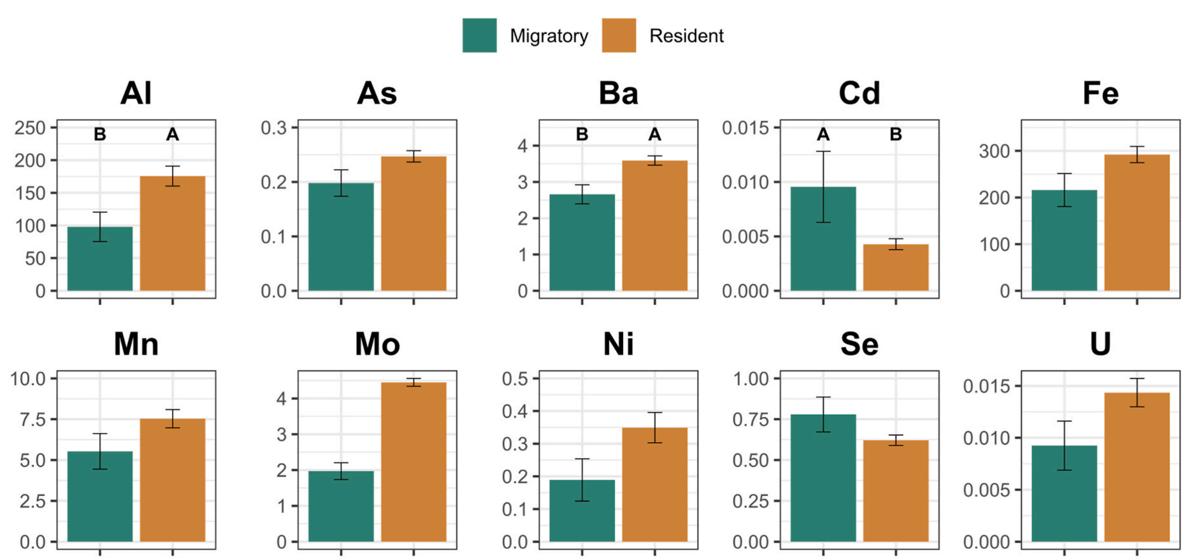

Fig. 7. Estimated marginal means (±SEM) of trace elements in bird feathers (in μg g−1 of dry wt.) by their migration patterns calculated based on GLM models with Al as a covariate. The results were averaged over the levels of feeding habits, study site, and age of feathers. Different letters above bars denote significant differences between groups ($p \leq 0.05$).

and Cristol, 2017). We compared our results with suggested threshold levels of toxic elements in feathers that correspond with different sublethal effects in birds (Burger and Gochfeld, 1997; Jackson et al., 2011; Ma et al., 2018). Ma et al. (2018) discovered that feather Hg higher than 1.43 μg g^{-1} may lead to reduced migration success and survival in blackpoll warbler (*Setophaga striata*). In our study, 28% (5/18) of birds, including barn swallow and common nightingale, had feather Hg higher than the aforementioned threshold. Our results revealed that 13% (9/69) of birds from Almadén had levels of Hg in feathers higher than a suggested threshold of 3 μg g^{-1}, which corresponds with reduced nest success in small passerines (Jackson et al., 2011). Those birds belonged to eight species including common kingfisher (N = 3), Cetti's warbler (N = 3), Iberian azure-winged magpie (N = 1), barn swallow (N = 1), common nightingale (N = 1), and European stonechat (N = 1). Amongst them, six individuals had Hg concentrations higher than 5 μg g^{-1}, which has been associated with adverse effects including reproductive disorders (Burger and Gochfeld, 1997).

We also found that Pb concentrations in the feathers of 14% (6/43) of Sierra Madrona birds and 4% (3/69) of Almadén birds were above a threshold of 4 μg g^{-1}, which is known to be associated with various behavioral, cognitive, and health effects in seabirds (Burger and Gochfeld, 2000). The birds with feather Pb concentrations higher than the suggested threshold belonged to four species: Eurasian jay (N = 1), house sparrow (N = 3), Iberian azure-winged magpie (N = 3), and Spanish sparrow (N = 2). Our findings highlight the need for further research that will use bioindicator species with small home ranges to identify potential hotspots of Hg and Pb emission in mining areas that should be remediated to protect local fauna from overexposure to toxic metals.

4. Conclusions

Our work shows the broad spectrum of metals and metalloids present in the feathers of birds inhabiting two abandoned non-ferrous metal mining areas in Spain. Overall, our results contribute to a better understanding of the effects of metal and metalloid pollution in areas of former metal mining on local avifauna. Despite some limitations, we indicated which ecological traits influence the concentrations of the studied elements in feathers. Our results confirmed that the concentrations of the majority of elements in feathers are largely influenced by their feeding habits, the age of feathers, and migration status. We also found differences in the elemental contents of feathers between the mining areas and the control area, indicating that feathers can serve as reliable passive bioindicators of habitat pollution. Analyzing the elemental content of feathers, it is crucial to keep in mind that despite thorough cleaning of feathers, external contaminants may persist and affect the interpretation of analytical results. Our data reveal continued exposure to Hg and Pb in birds living in the Almadén and Sierra Madrona mining areas with abandoned mines and spoil dumps, respectively. These defunct mine sites have never been remediated. We believe that much effort is still needed to secure these areas to protect local wildlife from the exposure to toxic elements.

5. Ethics

The study was approved by the Local Committee on Ethics and Animal Experimentation of the University of Castilla-La Mancha (PR-2016-02-03) and the authorization for the scientific capture of birds by the Junta de Comunidades de Castilla-La Mancha (DGPFEN/SEN/avp_16_024,178/17) and carried out according to the current Spanish and EU regulations on the use of animals in research. Mist netting, handling, ringing of birds were performed by authorized and experienced ornithologists according to the best practice and following the Guidelines to the Use of Wild Birds in Research (Fair et al., 2010).

Credit author statement

Maciej Durkalec: Writing - original draft, Investigation, Formal analysis, Funding acquisition., Software, Visualization. **Mónica Martínez-Haro**: Investigation, Writing - review & editing. **Agnieszka Nawrocka**: Investigation, Writing – review & editing. **Jennifer Pareja-Carrera:** Investigation. **Judit E.G. Smits**: Investigation, Writing – review & editing. **Rafael Mateo**: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.113575.

References

Abbasi, N.A., Jaspers, V.L.B., Chaudhry, M.J.I., Ali, S., Malik, R.N., 2015. Influence of taxa, trophic level, and location on bioaccumulation of toxic metals in bird's feathers: a preliminary biomonitoring study using multiple bird species from Pakistan. Chemosphere 120, 527–537. https://doi.org/10.1016/j.chemosphere.2014.08.054.

Abeysinghe, K.S., Qiu, G., Goodale, E., Anderson, C.W.N., Bishop, K., Evers, D.C., Goodale, M.W., Hintelmann, H., Liu, S., Mammides, C., Quan, R.C., Wang, J., Wu, P., Xu, X.H., Yang, X.D., Feng, X., 2017. Mercury flow through an Asian rice-based food web. Environ. Pollut. 229, 219–228. https://doi.org/10.1016/j.envpol.2017.05.067.

Ackerman, J.T., Hartman, C.A., Herzog, M.P., 2019. Mercury contamination in resident and migrant songbirds and potential effects on body condition. Environ. Pollut. 246, 797–810. https://doi.org/10.1016/j.envpol.2018.11.060.

Alleva, E., Francia, N., Pandolfi, M., De Marinis, A.M., Chiarotti, F., Santucci, D., 2006. Organochlorine and heavy-metal contaminants in wild mammals and birds of Urbino-Pesaro Province, Italy: an analytic overview for potential bioindicators. Arch. Environ. Contam. Toxicol. 51, 123–134. https://doi.org/10.1007/s00244-005-0218-1.

Aloupi, M., Ferentinou, E., Zaharaki, O.M., Akriotis, T., 2020. Does dilute nitric acid improve the removal of exogenous heavy metals from feathers? A comparative study towards the optimization of the cleaning procedure of feather samples prior to metal analysis. Ecotoxicol. Environ. Saf. 200, 110759. https://doi.org/10.1016/j.ecoenv.2020.110759.

Álvarez, C.R., Jiménez-Moreno, M., Bernardo, F.J.G., Martín-Doimeadios, R.C.R., Nevado, J.J.B., 2018. Using species-specific enriched stable isotopes to study the effect of fresh mercury inputs in soil-earthworm systems. Ecotoxicol. Environ. Saf. 147, 192–199. https://doi.org/10.1016/j.ecoenv.2017.08.015.

Atwell, L., Hobson, K.A., Welch, H.E., 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Can. J. Fish. Aquat. Sci. 55, 1114–1121. https://doi.org/10.1139/f98-001.

Beaubien, G.B., Olson, C.I., Todd, A.C., Otter, R.R., 2020. The spider exposure pathway and the potential risk to arachnivorous birds. Environ. Toxicol. Chem. 39, 2314–2324. https://doi.org/10.1002/etc.4848.

Becker, P.H., 2003. Chapter 19 biomonitoring with birds. In: Markert, B.A., Breure, A.M., Zechmeister, H.G. (Eds.), Trace Metals and Other Contaminants in the Environment. Elsevier, pp. 677–736. https://doi.org/10.1016/S0927-5215(03)80149-2.

Bennett, R., Hoff, D., Etterson, M., 2011. Assessment of Methods for Estimating Risk to Birds from Ingestion of Contaminated Grit Particles (No. OH. EPA/600/R-11/023) (Cincinnati).

Berglund, Åsa M.M., Koivula, Miia J., Eeva, Tapio, 2011. Species- and age-related variation in metal exposure and accumulation of two passerine bird species. Environ. Pollut. 159 (10), 2368–2374. https://doi.org/10.1016/j.envpol.2011.07.001.

Berzas Nevado, J.J., García Bermejo, L.F., Rodríguez Martín-Doimeadios, R.C., 2003. Distribution of mercury in the aquatic environment at Almadén, Spain. Environ. Pollut. 122, 261–271. https://doi.org/10.1016/S0269-7491(02)00290-7.

Best, L.B., Stafford, T.R., 2002. Influence of daily grit consumption rate and diet on gizzard grit counts. J. Wildl. Manag. 66, 381. https://doi.org/10.2307/3803171.

Beyer, W.N., Spann, J., Day, D., 1999. Metal and sediment ingestion by dabbling ducks. Sci. Total Environ. 231, 235–239. https://doi.org/10.1016/S0048-9697(99)00098-4.

Birds of the world. In: Billerman, S.M., Keeney, B.K., Rodewald, P.G., Schulenberg, T.S. (Eds.), Cornell Lab. Ornithol. [WWW Document]. URL https://birdsoftheworld.org/bow/home accessed 8.17.20.

Bond, Alexander L., 2010. Relationships between stable isotopes and metal contaminants in feathers are spurious and biologically uninformative. Environ. Pollut. 158 (5), 1182–1184. https://doi.org/10.1016/j.envpol.2010.01.004.

Borghesi, F., Dinelli, E., Migani, F., Béchet, A., Rendón-Martos, M., Amat, J.A., Sommer, S., Gillingham, M.A.F., 2017. Assessing environmental pollution in birds: a new methodological approach for interpreting bioaccumulation of trace elements in feather shafts using geochemical sediment data. Methods Ecol. Evol. 8, 96–108. https://doi.org/10.1111/2041-210X.12644.

Borghesi, F., Migani, F., Andreotti, A., Baccetti, N., Bianchi, N., Birke, M., Dinelli, E., 2016. Metals and trace elements in feathers: a geochemical approach to avoid misinterpretation of analytical responses. Sci. Total Environ. 544, 476–494. https://doi.org/10.1016/j.scitotenv.2015.11.115.

Bottini, C.L.J., MacDougall-Shackleton, S.A., Branfireun, B.A., Hobson, K.A., 2021. Feathers accurately reflect blood mercury at time of feather growth in a songbird. Sci. Total Environ. 775, 145739. https://doi.org/10.1016/j.scitotenv.2021.145739.

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. In: Hodgson, E. (Ed.), Reviews in Environmental Toxicology, vol. 5. Toxicology Communications, Inc., Raleigh, NC, pp. 203–311.

Burger, J., Gochfeld, M., 2000. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. Sci. Total Environ. 257, 37–52. https://doi.org/10.1016/S0048-9697(00)00496-4.

Burger, Joanna, 2002. Food chain differences affect heavy metals in bird eggs in Barnegat Bay, New Jersey. Environ. Res. 90 (1), 33–39. https://doi.org/10.1006/enrs.2002.4381.

Burger, J., Gochfeld, M., 1997. Risk, mercury levels, and birds: relating adverse laboratory effects to field biomonitoring. Environ. Res. 75, 160–172. https://doi.org/10.1006/enrs.1997.3778.

Burger, J., Gochfeld, M., Sullivan, K., Irons, D., McKnight, A., 2008. Arsenic, cadmium, chromium, lead, manganese, mercury, and selenium in feathers of Black-legged Kittiwake (Rissa tridactyla) and Black Oystercatcher (Haematopus bachmani) from Prince William Sound, Alaska. Sci. Total Environ. 398, 20–25. https://doi.org/10.1016/j.scitotenv.2008.02.051.

Camizuli, E., Scheifler, R., Garnier, S., Monna, F., Losno, R., Gourault, C., Hamm, G., Lachiche, C., Delivet, G., Chateau, C., Alibert, P., 2018. Trace metals from historical mining sites and past metallurgical activity remain bioavailable to wildlife today. Sci. Rep. 8, 3436. https://doi.org/10.1038/s41598-018-20983-0.

Candeias, C., Ávila, P., Coelho, P., Teixeira, J.P., 2019. Mining activities: health impacts. In: Encyclopedia of Environmental Health. Elsevier, pp. 415–435. https://doi.org/10.1016/B978-0-12-409548-9.11056-5.

Cardiel, I.E., Taggart, M.A., Mateo, R., 2011. Using Pb-Al ratios to discriminate between internal and external deposition of Pb in feathers. Ecotoxicol. Environ. Saf. 74, 911–917. https://doi.org/10.1016/j.ecoenv.2010.12.015.

Čech, M., Čech, P., 2015. Non-fish prey in the diet of an exclusive fish-eater: the Common Kingfisher Alcedo atthis. Hous. Theor. Soc. 62, 457–465. https://doi.org/10.1080/00063657.2015.1073679.

Chapa-Vargas, L., Mejía-Saavedra, J.J., Monzalvo-Santos, K., Puebla-Olivares, F., 2010. Blood lead concentrations in wild birds from a polluted mining region at Villa de la Paz, San Luis Potosí, Mexico. J. Environ. Sci. Health Part A Toxic/Hazard. Subst. Environ. Eng. 45, 90–98. https://doi.org/10.1080/10934520903389242.

Cooper, Z., Bringolf, R., Cooper, R., Loftis, K., Bryan, A.L., Martin, J.A., 2017. Heavy metal bioaccumulation in two passerines with differing migration strategies. Sci. Total Environ. 592, 25–32. https://doi.org/10.1016/j.scitotenv.2017.03.055.

da Silva-Júnior, A.H.M., da Silva, D.N., Ferrari, S.L.P., 2014. Mdscore : an R package to compute improved score tests in generalized linear models. J. Stat. Software 61. https://doi.org/10.18637/jss.v061.c02.

Dauwe, T., Bervoets, L., Blust, R., Eens, M., Dauwe, L., Bervoets, R., Blust, M., Ee, T., 2002. Tissue levels of lead in experimentally exposed zebra finches (Taeniopygia guttata) with particular attention on the use of feathers as biomonitors. Arch. Environ. Contam. Toxicol. 42, 88–92. https://doi.org/10.1007/s002440010295.

Downs, C.T., Bredin, I.P., Wragg, P.D., 2019. More than eating dirt: a review of avian geophagy. Afr. Zool. 54, 1–19. https://doi.org/10.1080/15627020.2019.1570335.

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol. 47, 4967–4983. https://doi.org/10.1021/es305071v.

Durkalec, M., Nawrocka, A., Krzysiak, M., Larska, M., Kmiecik, M., Posyniak, A., 2018. Trace elements in the liver of captive and free-ranging European bison (Bison bonasus L.). Chemosphere 193, 454–463. https://doi.org/10.1016/j.chemosphere.2017.11.050.

Eeva, T., Holmström, H., Espín, S., Sánchez-Virosta, P., Klemola, T., 2018. Leaves, berries and herbivorous larvae of bilberry Vaccinium myrtillus as sources of metals in food chains at a Cu-Ni smelter site. Chemosphere 210, 859–866. https://doi.org/10.1016/j.chemosphere.2018.07.099.

Ek, K.H., Morrison, G.M., Lindberg, P., Rauch, S., 2004. Comparative tissue distribution of metals in birds in Sweden using ICP-MS and laser ablation ICP-MS. Arch. Environ. Contam. Toxicol. 47, 259–269. https://doi.org/10.1007/s00244-004-3138-6.

Fair, J.M., Paul, E., Jones, J. (Eds.), 2010. Guidelines to the Use of Wild Birds in Research, third ed. The Ornithological Council, Washington, D.C.

Fritsch, C., Coeurdassier, M., Faivre, B., Baurand, P.E., Giraudoux, P., van den Brink, N. W., Scheifler, R., 2012. Influence of landscape composition and diversity on contaminant flux in terrestrial food webs: a case study of trace metal transfer to European blackbirds Turdus merula. Sci. Total Environ. 432, 275–287. https://doi.org/10.1016/j.scitotenv.2012.06.004.

Furness, R.W., Greenwood, J.J.D., 1996. Birds as Monitors of Environmental Change, Colonial Waterbirds. https://doi.org/10.2307/1521825.

Gil-Jiménez, E., Mateo, R., de Lucas, M., Ferrer, M., 2020. Feathers and hair as tools for non-destructive pollution exposure assessment in a mining site of the Iberian Pyrite Belt. Environ. Pollut. 263 https://doi.org/10.1016/j.envpol.2020.114523.

Gionfriddo, J.P., Best, L.B., 1996. Grit-use patterns in North American birds: the influence of diet, body size, and gender. Wilson Bull. 108, 685–696.

Gray, J.E., Hines, M.E., Higueras, P.L., Adatto, I., Lasorsa, B.K., 2004. Mercury speciation and microbial transformations in mine wastes, stream sediments, and surface waters at the Almadén Mining District, Spain. Environ. Sci. Technol. 38, 4285–4292. https://doi.org/10.1021/es040359d.

Hahn, E., Hahn, K., Stoeppler, M., 1993. Bird feathers as bioindicators in areas of the German environmental specimen bank - bioaccumulation of mercury in food chains and exogenous deposition of atmospheric pollution with lead and cadmium. Sci. Total Environ. 139–140, 259–270. https://doi.org/10.1016/0048-9697(93)90025-2.

He, C., Su, T., Liu, S., Jiang, A., Goodale, E., Qiu, G., 2020. Heavy metal, arsenic, and selenium concentrations in bird feathers from a region in southern China impacted by intensive mining of nonferrous metals. Environ. Toxicol. Chem. 39, 371–380. https://doi.org/10.1002/etc.4622.

Higueras, P., Esbrí, J.M., García-Ordiales, E., González-Corrochano, B., López-Berdonces, M.A., García-Noguero, E.M., Alonso-Azcárate, J., Martínez-Coronado, A., 2017. Potentially harmful elements in soils and holm-oak trees (Quercus ilex L.) growing in mining sites at the Valle de Alcudia Pb-Zn district (Spain)–Some clues on plant metal uptake. J. Geochem. Explor. 182, 166–179. https://doi.org/10.1016/j.gexplo.2016.07.017.

Higueras, P., María Esbrí, J., Oyarzun, R., Llanos, W., Martínez-Coronado, A., Lillo, J., Angel López-Berdonces, M., Maria García-Noguero, E., 2013. Industrial and natural sources of gaseous elemental mercury in the Almadén district (Spain): an updated report on this issue after the ceasing of mining and metallurgical activities in 2003 and major land reclamation works. Environ. Res. 125, 197–208. https://doi.org/10.1016/j.envres.2012.10.011.

Higueras, P., Oyarzun, R., Biester, H., Lillo, J., Lorenzo, S., 2003. A first insight into mercury distribution and speciation in soils from the Almadén mining district, Spain. J. Geochem. Explor. 80, 95–104. https://doi.org/10.1016/S0375-6742(03)00185-7.

Higueras, P., Oyarzun, R., Lillo, J., Sánchez-Hernández, J.C., Molina, J.A., Esbrí, J.M., Lorenzo, S., 2006. The Almadén district (Spain): anatomy of one of the world's largest Hg-contaminated sites. Sci. Total Environ. 356, 112–124. https://doi.org/10.1016/j.scitotenv.2005.04.042.

Higueras, P.L., Mansilla Plaza, L., Lorenzo Álvarez, S., Esbrí Víctor, J.M., 2011. The Almadén mercury mining district. Hist. Res. Miner. Resour. 13, 75–88.

Hildebrand, S.G., Huckabee, J.W., Diaz, F.S., Janzen, S.A., Solomon, J.A., Kumar, K.D., 1980. Distribution of Mercury in the Environment at Almaden, Spain. Environmental Science Division, Oak Ridge, TN. https://doi.org/10.2172/5008706 (United States).

Hui, C.A., 2004. Geophagy and potential contaminant exposure for terrestrial vertebrates. Rev. Environ. Contam. Toxicol. 183, 115–134. https://doi.org/10.1007/978-1-4419-9100-3_5.

Jackson, A.K., Evers, D.C., Etterson, M.A., Condon, A.M., Folsom, S.B., Detweiler, J., Schmerfeld, J., Cristol, D.A., 2011. Mercury exposure affects the reproductive success of a free-living terrestrial songbird, the Carolina Wren (Thryothorus ludovicianus). Auk 128, 759–769. https://doi.org/10.1525/auk.2011.11106.

Janssens, E., Dauwe, T., Bervoets, L., Eens, M., 2001. Heavy metals and selenium in feathers of great tits (Parus major) along a pollution gradient. Environ. Toxicol. Chem. 20, 2815–2820. https://doi.org/10.1002/etc.5620201221.

Jaspers, V., Dauwe, T., Pinxten, R., Bervoets, L., Blust, R., Eens, M., 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, Parus major. J. Environ. Monit. 6, 356. https://doi.org/10.1039/b314919f.

Jaspers, V.L.B., Covaci, A., Herzke, D., Eulaers, I., Eens, M., 2019. Bird feathers as a biomonitor for environmental pollutants: prospects and pitfalls. TrAC Trends Anal. Chem. (Reference Ed.) 118, 223–226. https://doi.org/10.1016/j.trac.2019.05.019.

Joffre, R., Vacher, J., de los Llanos, C., Long, G., 1988. The dehesa: an agrosilvopastoral system of the Mediterranean region with special reference to the Sierra Morena area of Spain. Agrofor. Syst. 6, 71–96. https://doi.org/10.1007/bf02220110.

Kabata-Pendias, A., Mukherjee, A.B., 2007. Trace Elements from Soil to Human, first ed. Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-32714-1.

Kassambara, A., 2020. ggpubr: "ggplot2" Based Publication Ready Plots. R package version 0.3.0 [WWW Document]. URL. https://cran.r-project.org/package=ggpubr.

Kassambara, A., Mundt, F., 2020. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.7 [WWW Document]. URL. accessed 7.1.20. https://cran.r-project.org/package=factoextra.

Kidd, K., Clayden, M., Jardine, T., 2012. Bioaccumulation and biomagnification of mercury through food webs. In: Liu, G., Cai, Y., O'Driscoll, N. (Eds.), Environmental Chemistry and Toxicology of Mercury. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 453–499. https://doi.org/10.1002/9781118146644.ch14.

Laskowski, R., Hopkin, S.P., 1996. Accumulation of Zn, Cu, Pb and Cd in the garden snail (Helix aspersa): implications for predators. Environ. Pollut. 91, 289–297. https://doi.org/10.1016/0269-7491(95)00070-4.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR : an R package for multivariate analysis. J. Stat. Software 25, 1–18. https://doi.org/10.18637/jss.v025.i01.

Lenth, R., 2020. Emmeans: estimated marginal means, aka least-squares means. R package version 1.4.7 [WWW Document]. URL. https://cran.r-project.org/package=emmeans. accessed 7.1.20.

Leonzio, C., Bianchi, N., Gustin, M., Sorace, A., Ancora, S., 2009. Mercury, lead and copper in feathers and excreta of small passerine species in relation to foraging guilds and age of feathers. Bull. Environ. Contam. Toxicol. 83, 693–697. https://doi.org/10.1007/s00128-009-9789-2.

Lester, M.B., Van Riper, C., 2014. The distribution and extent of heavy metal accumulation in song sparrows along Arizona's upper Santa Cruz River. Environ. Monit. Assess. 186, 4779–4791. https://doi.org/10.1007/s10661-014-3737-2.

Lopes, I., Sedlmayr, A., Moreira-Santos, M., Moreno-Garrido, I., Blasco, J., Ribeiro, R., 2010. European bee-eater (Merops apiaster) populations under arsenic and metal stress: evaluation of exposure at a mining site. Environ. Monit. Assess. 161, 237–245. https://doi.org/10.1007/s10661-008-0741-4.

Ma, Y., Hobson, K.A., Kardynal, K.J., Guglielmo, C.G., Branfireun, B.A., 2021. Inferring spatial patterns of mercury exposure in migratory boreal songbirds: combining feather mercury and stable isotope (δ2H) measurements. Sci. Total Environ. 762 https://doi.org/10.1016/j.scitotenv.2020.143109.

Ma, Y., Perez, C.R., Branfireun, B.A., Guglielmo, C.G., 2018. Dietary exposure to methylmercury affects flight endurance in a migratory songbird. Environ. Pollut. 234, 894–901. https://doi.org/10.1016/j.envpol.2017.12.011.

Markowski, M., Kaliński, A., Skwarska, J., Wawrzyniak, J., Bańbura, M., Markowski, J., Zieliński, P., Bańbura, J., 2013. Avian feathers as bioindicators of the exposure to heavy metal contamination of food. Bull. Environ. Contam. Toxicol. 91, 302–305. https://doi.org/10.1007/s00128-013-1065-9.

McClelland, S.C., Durães Ribeiro, R., Mielke, H.W., Finkelstein, M.E., Gonzales, C.R., Jones, J.A., Komdeur, J., Derryberry, E., Saltzberg, E.B., Karubian, J., 2019. Sub-lethal exposure to lead is associated with heightened aggression in an urban songbird. Sci. Total Environ. 654, 593–603. https://doi.org/10.1016/j.scitotenv.2018.11.145.

Ortiz-Santaliestra, M.E., Rodríguez, A., Pareja-Carrera, J., Mateo, R., Martinez-Haro, M., 2019. Tools for non-invasive sampling of metal accumulation and its effects in Mediterranean pond turtle populations inhabiting mining areas. Chemosphere 231, 194–206. https://doi.org/10.1016/j.chemosphere.2019.05.082.

Palero-Fernández, F.J., Martín-Izard, A., 2005. Trace element contents in galena and sphalerite from ore deposits of the Alcudia Valley mineral field (Eastern Sierra Morena, Spain). J. Geochem. Explor. 86, 1–25. https://doi.org/10.1016/j.gexplo.2005.03.001.

Pareja-Carrera, J., Mateo, R., Rodríguez-Estival, J., 2014. Lead (Pb) in sheep exposed to mining pollution: implications for animal and human health. Ecotoxicol. Environ. Saf. 108, 210–216. https://doi.org/10.1016/j.ecoenv.2014.07.014.

Patiño Ropero, M.J., Rodríguez Fariñas, N., Krupp, E., Mateo, R., Berzas Nevado, J.J., Rodríguez Martín-Doimeadios, R.C., 2016. Mercury and selenium binding biomolecules in terrestrial mammals (Cervus elaphus and Sus scrofa) from a mercury exposed area. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1022, 159–166. https://doi.org/10.1016/j.jchromb.2016.04.003.

Pilastro, A., Congiu, L., Tallandini, L., Turchetto, M., 1993. The use of bird feathers for the monitoring of cadmium pollution. Arch. Environ. Contam. Toxicol. 24, 355–358. https://doi.org/10.1007/BF01128733.

R Core Team, 2020. R: A language and environment for statistical computing. Version 4.0.2 [WWW Document]. Found. Stat. Comput. Vienna, Austria. accessed 7.5.20. https://www.r-project.org.

Ramírez, F., Forero, M.G., Hobson, K.A., Chiaradia, A., 2015. Older female little penguins Eudyptula minor adjust nutrient allocations to both eggs. J. Exp. Mar. Biol. Ecol. 468, 91–96. https://doi.org/10.1016/j.jembe.2015.03.020.

Reglero, M.M., Monsalve-González, L., Taggart, M.A., Mateo, R., 2008. Transfer of metals to plants and red deer in an old lead mining area in Spain. Sci. Total Environ. 406, 287–297. https://doi.org/10.1016/j.scitotenv.2008.06.001.

Reglero, M.M., Taggart, M.A., Monsalve-González, L., Mateo, R., 2009. Heavy metal exposure in large game from a lead mining area: effects on oxidative stress and fatty acid composition in liver. Environ. Pollut. 157, 1388–1395. https://doi.org/10.1016/j.envpol.2008.11.036.

Rodríguez-Estival, J., Morales-Machuca, C., Pareja-Carrera, J., Ortiz-Santaliestra, M.E., Mateo, R., 2019. Food safety risk assessment of metal pollution in crayfish from two historical mining areas: accounting for bioavailability and cooking extractability. Ecotoxicol. Environ. Saf. 185, 109682. https://doi.org/10.1016/j.ecoenv.2019.109682.

Rodríguez-Estival, J., Ortiz-Santaliestra, M.E., Mateo, R., 2020. Assessment of ecotoxicological risks to river otters from ingestion of invasive red swamp crayfish in metal contaminated areas: use of feces to estimate dietary exposure. Environ. Res. 181, 108907. https://doi.org/10.1016/j.envres.2019.108907.

Rodríguez-Estival, J., Pareja-Carrera, J., Mateo, R., 2014. Lead poisoning in a calf from the mining area of Sierra Madrona and Alcudia Valley. Rev. Toxicol. 31, 47–49.

Ruelas-Inzunza, J., Páez-Osuna, F., 2004. Trace metals in tissues of resident and migratory birds from a lagoon associated with an agricultural drainage basin (SE Gulf of California). Arch. Environ. Contam. Toxicol. 47, 117–125. https://doi.org/10.1007/s00244-004-3101-6.

Rybicka, E.H., 1996. Impact of mining and metallurgical industries on the environment in Poland. Appl. Geochem. 11, 3–9.

Sánchez-Virosta, P., Espín, S., García-Fernández, A.J., Eeva, T., 2015. A review on exposure and effects of arsenic in passerine birds. Sci. Total Environ. 512 (513), 506–525. https://doi.org/10.1016/j.scitotenv.2015.01.069.

Sánchez-Virosta, P., León-Ortega, M., Calvo, J.F., Camarero, P.R., Mateo, R., Zumbado, M., Luzardo, O.P., Eeva, T., García-Fernández, A.J., Espín, S., 2020. Blood concentrations of 50 elements in Eagle owl (Bubo bubo) at different contamination scenarios and related effects on plasma vitamin levels. Environ. Pollut. 265 https://doi.org/10.1016/j.envpol.2020.115012.

Sanderfoot, O.V., Holloway, T., 2017. Air pollution impacts on avian species via inhalation exposure and associated outcomes. Environ. Res. Lett. 12 https://doi.org/10.1088/1748-9326/aa8051.

Saupe, F., 1990. Geology of the almaden mercury deposit, province of Ciudad real, Spain. Econ. Geol. 85, 482–510. https://doi.org/10.2113/gsecongeo.85.3.482.

Scheuhammer, Anton M., Meyer, Michael W., Sandheinrich, Mark B., Murray, Michael W., 2007. Ambio 36 (1), 12–18. https://doi.org/10.1579/0044-7447(2007)36[12:EOEMOT]2.0.CO;2.

Sierra-Marquez, L., Peñuela-Gomez, S., Franco-Espinosa, L., Gomez-Ruiz, D., Diaz-Nieto, J., Sierra-Marquez, J., Olivero-Verbel, J., 2018. Mercury levels in birds and small rodents from Las Orquideas National natural park, Colombia. Environ. Sci. Pollut. Res. 25, 35055–35063. https://doi.org/10.1007/s11356-018-3359-2.

Svensson, L., 1992. Identification Guide to European Passerines, fourth ed. Lars Svensson, Stockholm.

Tejero, J., Higueras, P.L., Garrido, I., Esbrí, J.M., Oyarzun, R., Español, S., 2015. An estimation of mercury concentrations in the local atmosphere of Almadén (Ciudad Real Province, South Central Spain) during the twentieth century. Environ. Sci. Pollut. Res. 22, 4833–4841. https://doi.org/10.1007/s11356-014-2860-5.

Vallverdú-Coll, N., Mateo, R., Mougeot, F., Ortiz-Santaliestra, M.E., 2019. Immunotoxic effects of lead on birds. Sci. Total Environ. 689, 505–515. https://doi.org/10.1016/j.scitotenv.2019.06.251.

Venkateswarlu, K., Nirola, R., Kuppusamy, S., Thavamani, P., Naidu, R., Megharaj, M., 2016. Abandoned metalliferous mines: ecological impacts and potential approaches for reclamation. Rev. Environ. Sci. Biotechnol. 15, 327–354. https://doi.org/10.1007/s11157-016-9398-6.

Wei, T., Simko, V., 2017. R Package "Corrplot": Visualization of a Correlation Matrix (Version 0.84) [WWW Document]. URL. accessed 5.8.19. https://github.com/taiyun/corrplot.

Weyers, B., Glück, E., Stoeppler, M., 1988. Investigation of the significance of heavy metal contents of blackbird feathers. Sci. Total Environ. 77, 61–67. https://doi.org/10.1016/0048-9697(88)90315-4.

Whitney, M.C., Cristol, D.A., 2017. Impacts of sublethal mercury exposure on birds: a detailed review. In: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology. Springer Berlin Heidelberg, Cham, pp. 113–163. https://doi.org/10.1007/398_2017_4.

Wickham, H., 2016. ggplot2, second ed. Use R! Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-24277-4.

Wickham, H., François, R., Henry, L., Müller, K., Romain, F., Lionel, H., Müller, K., 2020. Dplyr: A Grammar of Data Manipulation. R package version 1.0.2.

Williams, R.J., Holladay, S.D., Williams, S.M., Gogal, R.M., 2017. Environmental lead and wild birds: a review. In: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology. Springer International Publishing AG, Cham, pp. 157–180. https://doi.org/10.1007/398_2017_9.

Yap, B.W., Sim, C.H., 2011. Comparisons of various types of normality tests. J. Stat. Comput. Simulat. 81, 2141–2155. https://doi.org/10.1080/00949655.2010.520163.

Zolfaghari, G., Esmaili-Sari, A., Ghasempouri, S.M., Baydokhti, R.R., Hassanzade Kiabi, B., 2009. A multispecies-monitoring study about bioaccumulation of mercury in Iranian birds (Khuzestan to Persian Gulf): effect of taxonomic affiliation and trophic level. Environ. Res. 109, 830–836. https://doi.org/10.1016/j.envres.2009.07.001.