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Toxic elements in blood of red-necked nightjars (*Caprimulgus ruficollis*) inhabiting differently polluted environments

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Abstract

Toxic metals have been widely reported in avian tissues due to their well-known accumulation capacity and adverse effects. However, rare earth elements (REE) and other minor elements (ME) are becoming a new threat due to their use in modern technology. Presently, exposure data are limited and no studies have been reported in wildlife. The order Caprimulgiformes is among the most understudied groups of birds lacking blood ecotoxicological data. One major constraint is the small blood sample volume that can be collected to ensure animal welfare, which limits analyses. In order to shed light on these collective issues, we sampled 48 red-necked nightjars (*Caprimulgus ruficollis*) inhabiting three different scenarios of contaminant exposure (agricultural-urban area, n=15; mining area, n=17; and control area, n=16) in southeastern Spain, and report for the first time concentrations of 50 elements (i.e. trace elements, ATSDR's list toxic elements, REE and ME) using a recently developed technique able to analyze them by ICP-MS in very small volumes of blood (130 µL). Concentrations of As, Cd, Pb and Mn were significantly higher in individuals captured at the mining area compared to the other sites. Lead levels in the mine site were of particular concern since it was in the range of blood concentrations related to subclinical/clinical effects in other species, and in our study were associated with decreased hematocrit values (up to 44% hematocrit depression at blood concentrations >1000 ng/ml w.w.). Moreover, additive effects related to metal cocktail exposure in the mining area could be expected. Age and gender-related differences in blood concentrations were found for some elements. Even though most REE and ME concentrations were close to the LOQ, some of these emerging contaminants may trigger sublethal effects that, together with the ATSDR's list toxic elements, need to be carefully evaluated in a future study.

Capsule: First report of blood metal concentrations in Caprimulgiformes

Keywords: metal exposure; nightjars; *Caprimulgus ruficollis*; industrial emissions; urbanization

Introduction

Toxic elements have been widely reported in avian tissues due to their well-known bioaccumulation capacity and the adverse effects they cause in birds, being able to alter reproductive success and behavior, compromise immune function and affect different biochemical parameters (Eeva et al., 2005; Espín et al., 2016a, 2016b; Pain et al., 2019; Sánchez-Virosta et al., 2015; Vallverdú-Coll et al., 2019; Whitney and Cristol, 2018). However, the scientific community agrees that further research evaluating metal exposure is required to assess spatial and temporal trends and fill the knowledge gap for some avian species, and even for entire orders of birds from which no data exist. In this way, biomonitoring studies can provide early warning of potential impacts in wildlife species and the wider environment, and can be used to track the success of mitigation in reducing exposure (Espín et al., 2016c).

Some metalloids and metals such as arsenic (As), lead (Pb), mercury (Hg) or cadmium (Cd) are clearly considered toxic and are ranked in the Substance Priority List (1^{st}, 2^{nd}, 3^{rd} and 7^{th} position, respectively) prepared by the Agency of Toxic Substances and Disease Registry (ATSDR, 2017). Rare earth elements (REE) and other minor elements (ME) are becoming increasingly important due to their intensive use in modern technology all over the world, generating aerial emissions and tons of electronic waste (Hussain and Mumtaz, 2014; Tansel, 2017). However, data on exposure to these elements have been rarely reported (e.g. in humans: Gaman et al., 2019; González-Antuña et al., 2017), with no studies in wildlife. Thus, there is much uncertainty about their accumulation capacity and the potential short and long-term consequences for wild avian populations. This may be of particular concern to those species migrating to developing/under developed countries receiving hazardous waste from more developed countries and where almost all the e-waste recycling chain reaches the informal sector (collection, manual dismantling, open burning to recover metals and open dumping of residuals), with the consequent impact on health and the environment (Tansel, 2017; UNEP, 2011).

Nightjars (Caprimulgiformes) are crepuscular and nocturnal insectivorous birds widespread over the world, including more than 120 species distributed on all continents except in artic climates and New Zealand (Cleere, 2010). Despite wide geographic distribution, nightjars are among the most poorly studied groups of birds, since their nocturnal habits, cryptic plumage and elusive behavior have traditionally hampered the study of their biology and ecology (Braun and Huddleston, 2009). Recently, information for a few species has increased due to the development of suitable monitoring and sampling methods (e.g. Camacho et al., 2016; Evens et al., 2017), but many general aspects of their relation with human activities and responses to habitat degradation remain still unknown. For example, to our knowledge, no studies have been conducted to explore the exposure and effects of environmental pollutants in any nightjar species, despite of the variety of pollutants emitted worldwide from a range of sources such as agriculture, industry and mining activities.

The Red-necked nightjar (*Caprimulgus ruficollis*, hereafter called "nightjar") is a long-distance migrant quite common in warm regions of the Iberian Peninsula and northern Africa, where it breeds from early spring to late summer (Aragonés, 2003; Camacho, 2013a). Until few years ago, this species has been considered one of the least known birds in its distribution area (Aragonés, 2003; Sáez-Gómez et al., 2015). Wintering grounds are located on Western Sahara, presumably in countries such as Mali, Guinea Bissau and Senegal, although the exact range is unclear (Cleere et al., 2013). From dusk to dawn, nightjars use open shrublands, crops and roads to hunt aerial insects through short flights or taking prey directly from the ground (Camacho, 2013b; Jackson, 2003). Main prey species of nightjars are nocturnal insects belonging to orders Lepidoptera, Homoptera and Neuroptera (Camacho, 2013a), although its diet has not been thoroughly explored. In Spain, field observations have reported moths (Noctuidae and Thaumetopoeidae families, among others) as the main species composing the nightjar diet (Sáez and Camacho, 2016), however, other insect families such as Gryllidae and Cicadidae can contribute significantly to nightjar diet when their populations peak (authors' unpublished data). Nightjars show a notable tolerance to anthropogenic alterations, being able to reach high population densities even in highly managed landscapes (Camacho et al., 2014). Due to this ecological plasticity, nightjars can occur from natural Mediterranean shrublands to mining-impacted areas or intensive irrigation crops. However, toxic elements exposure and potential effects from industrial activities (i.e. mining exploitation or intensive agriculture) on nightjar physiology and fitness condition have never been studied. Hence, this study aims to evaluate the exposure to 50 elements (i.e. trace elements, ATSDR's list toxic elements, REE and ME) in nightjars inhabiting three different scenarios of contaminant exposure in the southeast of Iberian Peninsula. We expect increased Pb concentrations in the mining area based on previous findings in other species, showing δALAD inhibition (Espín et al., 2015) that may lead to pathologic effects including anemia, reduced hematocrit and hemoglobin or even brain damage (Dieter and Finley, 1979; Hoffman et al., 2000). Thus, potential effects of Pb exposure on hematocrit levels are also tested. Finally, we also evaluate the influence of factors at individual level, such as age and sex, on the observed concentrations of these elements in whole blood.

Material and methods

Study area

This study was conducted in the province of Murcia, in the southeast of Iberian Peninsula (37º 45´N, 0º 57´W) (Figure 1). The study area is characterized by a Mediterranean semi-arid climate, with a strong water deficit during spring and summer and scarce rainfall occurring predominantly in winter. Main land use of the study area is irrigation agriculture, which extends over lowlands, while highland landscapes are dominated by a mosaic of rainfed crops, pine forests and Mediterranean shrublands.

Three different scenarios of contaminant exposure were selected in the study area according to the main land use in each one: agricultural-urban area, mining area and control area. Agricultural-urban area is located at the center of the province of Murcia, next to Guadalentín River, and is occupied by large extensions of lucerne and cotton crops adjacent to an industrial park and a densely populated city. Contamination sources in this area are not known, but it is expected that intense agriculture and industrial activities lead to moderate pollution levels. Mining area corresponds to an ancient mining site (Cartagena-La Unión Mining District) located on the south of the province of Murcia, with extraction activity since Phoenicians, Carthagineans and Roman times until the end of the 20th century (ceased in 1992) (Conesa et al., 2008). Significant pollutant levels have been found in blood samples of wildlife in this area, mainly for metals such as Pb, Hg and Cd, among others (Espín et al., 2014a, 2014b; García-Fernández et al., 1995). Furthermore, toxic metals are still spread by small creeks from headwaters, due to the eroding process of runoff waters, impacting on surrounding ecosystems (Conesa and Schulin, 2010). Finally, we selected Escalona and Altaona mountains as a control area, where no contaminants are known to affect wildlife (Espín et al., 2014b). Human occupancy in this area is considerably low and landscape is dominated mainly by small tree crops (citric, almond and olive trees) scattered in a shrubland and forest matrix (León-

Ortega et al., 2017). Then, pollution sources are scarce and localized in this area (Espín et al., 2014a, 2014b).

Sampling and measurements

In June and July 2017, seven sampling nights were conducted in the three different pollution scenarios, and a total of 48 nightjars were captured (n=15 at agricultural-urban area, n=17 at mining area and n=16 at control area). From dusk to dawn, we drove a car at constant speed (15-20 km/h) over secondary roads distributed throughout the study areas. Nightjars were detected by the eye shine triggered when *tapetum lucidum* was lighting on frontal view. When detected, birds were captured using a torch and handheld net with 10 mm mesh-size (Camacho et al., 2014; Jackson, 2003). Once captured, each nightjar was individually marked with metal ring, and the age, sex, weight and other biometric measures (not used in this study) were recorded. Age and sex (n=19 males and n=29 females) were determined by feather design in wing and tail, following available literature for this species (Alonso and Caballero, 2003; Forero et al., 1995; Gargallo, 1994; Tornero and Sanchís, 2017). We grouped birds into three age classes: juveniles (young birds in their first summer – EURING age code III, n=5), breeding subadults (birds in their second summer – EURING age code V, n=8) or breeding adults (older birds – EURING age code VI, n=35).

The clinical examination of each bird was performed by a veterinarian prior to blood sampling. No symptoms were observed in the individuals sampled, being considered clinically healthy. Blood samples (approximately 1 mL) were collected by puncturing brachial veins with 30G needles and 1 mL-syringes and stored in heparinized Eppendorf tubes under refrigerated conditions until processed in the laboratory. Hematocrit (% of red blood cells from total sample volume) was recorded using capillary tube readers after centrifugation of blood at 2200 g for 5 min. One Eppendorf tube with whole blood was frozen at −80°C until element analysis. Other Eppendorf tube with whole blood was centrifugated to separate plasma and red blood cell, and the tubes were frozen at −80°C for biochemical analysis that will be the scope of a different publication. Duration of the handling process per sampled nightjar ranged from 10 to 15 minutes and all birds were released exactly in the same location where they were caught.

Trace element analysis

We determined whole blood concentrations of 50 elements (Table 1), which were selected according to their toxicity and/or their frequent use in the manufacture of electronic consumer products (Hussain and Mumtaz, 2014; Tansel, 2017). For the elemental analysis we employed an Agilent 7900 ICP-MS equipment (Agilent Technologies, Tokyo, Japan) equipped with standard nickel cones, Ultra High Matrix Introduction (UHMI) system, and a cross-flow nebulizer with a Make-Up Gas Port (X400 Nebulizer, Savillex Corporation, MN, USA). We followed a procedure developed for human blood, which had been previously validated in our laboratory using certified reference materials (González-Antuña et al., 2017). Briefly, 130 µL of blood were diluted using 1120 µL of ammonia solution (0.05% of EDTA, 0.05% of Triton X-100, and 1% of NH_4OH), and 50 µL of internal standards (ISTD) were added (final volume = 1.3 mL). ISTD solution consisted of Sc (scandium), Ge (germanium), Rh (rhodium), and Ir (iridium) at a stock concentration of 20 mg/mL each. Pure standards of elements in acid solution (5% HNO_3, 100 mg/L) were purchased from CPA Chem (Stara Zagora, Bulgaria). Two standard curves (ten points, 20 ng/mL – 0.005 ng/mL) were made to avoid interferences between elements: a) one using a commercial multi-element mixture (CPA Chem, 100 mg/L, 5% HNO_3) containing all the essential elements and main heavy metals; and b) other multi-element mixture tailor-made in our laboratory from individual elements (CPA Chem), which contained the REE and ME. The limits of quantification (LOQs) ranged between 0.005 and 1.0 ng/ml, and the accuracy of measurements were in the range of 79 − 138%, with relative standard deviations (RSD) below 6% in all cases, as previously described (González-Antuña et al., 2017).

Statistical procedures

For all 50 elements we first calculated ranges and quartiles (Table 1). Most of the elements showed some values below the limit of quantification (<LOQ) and for many of them this proportion was relatively high (Table 1). Therefore, for statistical comparison we only selected those elements where the proportion of <LOQ values was below 15% (EPA, 2000). For these 17 elements we substituted <LOQ values by a random number between 0 and LOQ. Three of the elements (Cu, Fe, Mo) were normally distributed (by visual inspection of histogram and Kolmogorov-Smirnov test for normality) while the rest of the elements were $\log_{10}$ transformed to make them better conform normal

distribution. After that, we analyzed differences among sampling areas by linear models (LM) with area, sex and age as explanatory factors. Because we found relatively high Pb values in nightjar blood we further tested the association between Pb level and hematocrit with a generalized linear model (GLM; using beta error distribution and logit link function) where Pb level was used as an explanatory factor together with sex and age). Model estimates (least squares means and confidence limits) for $\log_{10}$-transformed values were back-transformed to the original scale for tables and figures. For the elements that showed significant differences among areas or age classes we further ran Tukey's test adjusted for the number of pairwise comparisons. Alpha level was set to 0.05 in all analyses. Finally, associations among elements were inspected with a hierarchical cluster analysis by using Pearson correlation matrix with average linkage method for clustering. All the analyses were ran with a statistical software SAS 9.4 (SAS Institute Inc., 2013).

Results and discussion

Many of the elements analyzed (33 out of 50, mainly REE and ME) were below LOQ in more than 15% of the individuals (Table 1), most often indicating low general levels and suggesting limited effects at the population level. For those elements where the proportion of <LOQ values was below 15%, mean concentrations in whole blood of nightjars by sampling area and GLMs are shown in Table 2. In general, element concentrations did not differ among the areas. However, concentrations of As, Cd, Pb and Mn were significantly increased in blood of individuals captured at the mining area compared to the other sites (for Mn only compared to the agricultural-urban area), while Mo concentrations were reduced in nightjars from the mining area compared to the control site (Table 2).

Birds are widely used in biomonitoring studies to evaluate toxic element contamination from industrial activities, and insectivorous birds have proved to be useful to assess metal exposure because they accumulate metals through the diet (Berglund et al., 2011; Eeva et al., 2009; Espín et al., 2016d; Sánchez-Virosta et al., 2015). In this study, the diet is considered the main exposure route of toxic metals in nightjars (i.e. ingestion of prey that have accumulated the elements), as reported for other species inhabiting the same area (Espín et al., 2014a, 2014b) and in general in different bird studies (Espín et al., 2016c; Sánchez-Virosta et al., 2015). Field observations have reported that the diet of nightjars is mainly composed by nocturnal aerial insects, with moths representing the main prey species in Spain (Sáez and Camacho, 2016). In this sense, larvae of moths (Lepidoptera) have shown increased toxic element concentrations (e.g. As, Cd, Pb) in metal-polluted environments compared to reference sites (Eeva et al., 2018). However, as explained before, the diet of nightjars has not been thoroughly explored due to the difficulties of monitoring and capturing this species. Therefore, further studies are needed to explore their diet composition, as well as to develop suitable sampling methods for metal analyses in prey items.

Once absorbed, elements are transported and distributed throughout the organism via the blood, and their concentrations in internal tissues are a key indicator of bioaccumulation (Espín et al., 2016c). The main accumulation organ depends on the toxicokinetics of each element and element form as well as the type of exposure: e.g. bone is the main organ of Pb accumulation but there is also distribution to liver, kidney and brain; kidney and liver are the main organs for Cd and Hg accumulation; liver is the main organ for As (Espín et al., 2016c; Sánchez-Virosta et al., 2015). The main excretion rate and route also depends on the element and species physiology, being urine and feces the main route, although some metals can be sequestered in feathers during molt or in the eggs/eggshells during laying (Espín et al., 2016c; Sánchez-Virosta et al., 2015). Overall, blood levels provide a non-destructive measure of contaminant exposure (Espín et al., 2016c). Blood is a proxy of short-term exposure to metals, and although it could reflect part of the metals accumulated in wintering grounds (for adults birds) and mobilized during breeding, it mostly reflects recent exposure in the breeding area (Espín et al., 2016c). Due to the lack of ecotoxicological data not only in the Caprimulgidae family, but in the whole Caprimulgiformes taxa, element concentrations found in this study were compared to those reported in different wild birds (mainly feeding on insects when possible). In this study, Pb is of particular concern due to the marked differences between the mining area and the other sites, nightjars in the mine site showing Pb concentrations ca. 43 ×X higher than in the control area. Previous studies developed during the 1990s and 2010s demonstrate that birds inhabiting close to this ancient mine site show higher Pb concentrations (Espín et al., 2014b, 2015; García-Fernández et al., 1995) due to the intensive mining activity (mainly of Pb, Zn, Cu, Mn, Fe, Ag and Sn) developed for more than 2500 years until its closing in 1992 (Conesa et al., 2008; Pavetti et al., 2006), being the principal Pb and Zn source in Spain during the 19th century (Estevan-Senís, 1967).

Blood Pb concentrations found in nightjars inhabiting the mining area (geometric mean = 165 ng/ml w.w.) were lower than those found in red-winged blackbirds (*Agelaius phoeniceus*), American robins (*Turdus migratorius*) and feral pigeon (*Columba livia*) at contaminated sites in USA (Cai and Calisi, 2016; Roux and Marra, 2007; Tsipoura et al., 2008) and similar to those found in northern mockingbird (*Mimus polyglottos*) and common blackbirds (*Turdus merula*) in USA and France, respectively (Roux and Marra, 2007; Scheifler et al., 2006) (Figure 2). However, in general, Pb concentrations in nightjars were higher than those reported in little owl (*Athene noctua*), common kestrel (*Falco tinnunculus*) and gray catbird (*Dumetella carolinensis*) inhabiting polluted environments in Spain and USA, the latter species exhibiting worse body condition as a results of Pb contamination (García-Fernández et al., 1995, 1997; Roux and Marra, 2007) (Figure 2).

Regarding other elements, As blood concentrations in the mining area were similar to those found in red-winged blackbirds at polluted sites in USA (Tsipoura et al., 2008), and Cd levels were lower than those reported in blackbirds, and similar to those found in common kestrel, little owl (García-Fernández et al., 1995), and nestling eagle owls (*Bubo bubo*) in the same mine site (Espín et al., 2014b). These As and Cd concentrations are within the range considered as low exposure levels in birds (Espín et al., 2014b; García-Fernández et al., 1995; Tsipoura et al., 2008). Both Mn and Mo are essential elements, but exposure to high levels may cause adverse effects (Stafford et al., 2016; Williams et al., 2012). Blood concentrations found in nightjars in the mining area were within the range of values found by other researchers in apparently healthy birds: Mo levels were similar to those reported in urban areas in France for great tit (*Parus major*) (19 ng/ml) (Bailly et al., 2017) and lower than those found in Northwestern crows (*Corvus caurinus*) in USA (55-66 ng/ml w.w.) (Van Hemert and Handel, 2016), while Mn concentrations were similar to those found in Northwestern crows (28-33 ng/ml w.w.) and much lower than those reported in African whitebacked vultures (*Pseudogyps africanus*) in South Africa (162-196 ng/ml w.w. in juveniles and 473 ng/ml w.w. in adults) (Van Hemert and Handel, 2016; van Wyk et al., 2001).

Pb levels in the mining area were in the range of blood concentrations related to subclinical signs in birds, 65% of individuals exceeding 200 ng/ml w.w. (considered the benchmark value for subclinical and physiological effects for Anseriformes and Falconiformes), 35% exceeding 500 ng/ml (considered clinical poisoning) and 2 individuals (12%) exceeding 1000 ng/ml in blood (related to severe clinical poisoning in Anseriformes and Falconiformes) (Franson and Pain, 2011). A previous study in the same mining area showed that nestling eagle owls exposed to Pb suffer up to 79% δALAD inhibition at blood Pb concentrations ≥ 190 ng/ml (Espín et al., 2015). Some researchers suggest that high inhibitions (>75%) for this enzyme may lead to pathologic effects including anemia, reduced hematocrit and hemoglobin or even brain damage (Dieter and Finley, 1979; Hoffman et al., 2000). We found that hematocrit of red-necked nightjars was negatively associated to blood Pb level (GLM: $F_{1,43} = 10.4$, $p = 0.0024$; estimate = −0.00052), but did not depend on age ($F_{2,43} = 1.0$, $p = 0.37$) or sex ($F_{1,43} = 1.6$, $p = 0.21$). Hematocrit values decreased by 4, 12 and 44% (hematocrit: 37, 34 and 22%) at blood concentrations ranging 200-500, 500-1000 and exceeding 1000 ng/ml, respectively, as compared with the mean hematocrit value (38%) in nightjars from the less Pb-polluted areas, with blood Pb concentrations averaging 12.8 ng/ml (maximum of 31.6 ng/ml). Hematocrit is a marker of general health (Boross et al., 2012), and its depression in some individuals of this study, possibly partly due to Pb exposure, may be indicative of deteriorated health condition. One study showed that a 94% decrease in δALAD activity was needed to cause a decline of 11% in hematocrit in griffon vulture (*Gyps fulvus*) (Espín et al., 2015), suggesting that nightjars showing high Pb concentrations could present an important δALAD enzyme inhibition. In addition, previous studies in the mining area showed that blood concentrations as low as 30 ng/ml for Pb or 0.2 ng/ml for Cd were able to deplete major antioxidants of the red blood cells (i.e. glutathione, glutathione peroxidase, catalase) and induce lipid damage in nestling eagle owls (Espín et al., 2014b). This suggests that the classical threshold level for Pb (and possibly for other elements) commonly accepted for considering physiological effects in certain avian species should be revised in the wild, where individuals are exposed to Pb but also to a combination of other contaminants and stressors. Therefore, potential sublethal effects of some elements, mainly Pb but also Cd, As and others, on nightjar's physiology cannot be excluded, and we consider that they will most likely occur. Moreover, additive effects related to metal cocktail exposure in the mining area could be expected.

Fallon et al. (2017) published some guidelines for evaluation of Pb poisoning of wild raptors, presenting the criteria for removing a Pb-poisoned bird from the wild for treatment: (i) if blood Pb concentration is low (≤ 400 ng/ml) and the bird appears healthy, it should be returned to the wild, (ii) if blood Pb concentration is elevated (400-600 ng/ml) but clinical signs are not present, the bird should be returned to the wild, but if clinical signs are present, the bird should be transferred to a rehabilitator for treatment, and (iii) if blood Pb concentrations are toxic (≥ 600 ng/ml), the bird should be transferred to the rehabilitator regardless of the symptoms. Chelation therapy is the main treatment for Pb poisoning, using chelation agents such as dimercaptosuccinic acid (succimer) and $CaNa_2$ ethylene diamine tetra acetic acid (CaEDTA) (Redig and Arent, 2008). Fallon et al. (2017) inform that these categories should be extrapolated with caution to different raptors species. Clinical effects of blood Pb concentrations varies among species: e.g. clinical signs in swans (*Cygnus* sp.) have been reported at ≥ 400 ng/ml and lethal values are > 2000 ng/ml (Degernes et al., 2002; Sears et al., 1989). Therefore, further studies are needed to understand how different species respond to Pb exposure (Fallon et al., 2017). This issue should be carefully evaluated in a future study to ascertain how nightjars' physiology and condition respond to Pb exposure. Sublethal effects of Pb exposure on breeding performance should also be considered. Pb-related impacts on avian reproduction have been well documented (e.g. reduced fertilization, hatching rate, breeding success, sperm motility) (Fritsch et al., 2019; Gasparik et al., 2012; Vallverdú-Coll et al., 2016). Efforts to monitor breeding and ecological parameters in nightjars in the study area have just started, thus reproductive data will be gathered in next years to evaluate population temporal changes and the potential reproductive consequences of Pb exposure.

Four elements (Cu, Mn, Mo and Sr) showed sex differences, females (all of them sampled in breeding season) showing higher concentrations than males (Figure 3). This species does not show sexual dimorphism in body size or body mass (Camacho et al., 2016; Forero et al., 1995), so this would not explain the gender-related differences found here. Cu, Mn and Mo are essential elements required during the egg development, which need mineral stores deposited in the egg at the time of its formation for the proper embryogenesis (Miles, 2000; Vieira, 2007). Thus, gender-related differences in the physiological state, with breeding females showing higher levels of some essential elements to be transferred to the egg, could explain this result. In addition, food intake of laying females could also be higher, increasing accumulation from the environment, or females in their reproductive period could choose prey with greater nutritional value (proteins, lipids, calcium, other essential elements, etc.), as occurs in other bird species. On the other hand, two elements (Cd, Hg) showed age-specific differences, with the lowest concentrations in the youngest age class (Figure 4). Increased concentrations in adults may be explained by the longer time to bioaccumulate and increase their metal body burden (Evers, 2018; Sánchez-Virosta et al., 2015; Wayland and Scheuhammer, 2011). In this sense, accumulation by age seems to be very typical especially for Cd, even in wild animals exposed to low background levels of dietary Cd (Berglund et al., 2011; Scheuhammer, 1987). In addition, although blood concentrations mainly reflect recent exposure (primarily via ingestion) (Burger et al., 2018; Espín et al., 2016c), they also partly reflect bioaccumulated elements that transfer from tissues to blood (Burger et al., 2018). Thus, since juveniles birds are in their first summer and they have not spent the winter in Africa yet, it is possible that their subadult/adult counterparts have accumulated higher levels of those specific metals in their wintering grounds (located on Western Sahara (Cleere et al., 2013). The greater effectiveness in finding food for adults compared to young birds, also increases the possibility of contaminant exposure in older birds. Finally, potential age or gender-related differences in diet could also explain these results. However, no studies on age or gender-related differences in feeding behavior and diet composition are available for this species.

The toxic elements Pb, Cd, As and Hg were grouped in a cluster analysis (Figure 5), indicating correlation among them and suggesting common origin in the polluted site. Correlations among the less toxic elements might reflect homeostatic regulation which controls absorption and hence body trace element concentrations (e.g. essentials elements like Co, Mn and Zn, or Se and Fe). However, the clusters generated could also be explained by other reasons such as diet composition, element similarities in atomic structure/reactivity, and soil composition (physicochemical factors may control element associations during soil formation, e.g. Co and Mn are closely associated in soils because their similar chemical properties, Bradford et al., 1996; Uren, 2013).

Conclusions

Due to the lack of ecotoxicological studies on Caprimulgiformes taxa, this article reports for the first time blood concentrations of 50 elements (i.e. trace elements, ATSDR's list toxic elements, REE and ME) in red-necked nightjars (*Caprimulgus ruficollis*) inhabiting three different scenarios of contaminant exposure, using a recently developed ICP-MS technique requiring small sample volumes. Blood As, Cd, Pb and Mn concentrations were significantly increased in nightjars captured at the mining area compared to the control and agricultural-urban areas. Lead levels in the mine site are of particular concern since they were in the range of blood concentrations related to subclinical and clinical effects in others avian taxa, and in our study were associated with decreased hematocrit values (up to 44% hematocrit depression at blood concentrations exceeding 1000 ng/ml w.w.). A few elements (Cu, Mn, Mo and Sr) showed higher values in females than in males, and two elements (Cd and Hg) accumulated with the age. It is well known that metals and metalloids such as Pb, Cd, Hg or As may produce oxidative stress and alter calcium metabolism. Thus, sublethal effects of some elements (mainly Pb) on nightjar's physiology with potential medium and long-term consequences cannot be discarded. Moreover, additive effects related to metal cocktail exposure in the mining area could be expected. Even though most REE and ME concentrations were close to the LOQ, some of these emerging contaminants may trigger sublethal effects that, together with the ATSDR's list toxic elements, needs to be evaluated in a future study.

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Tables

Table 1. Range and quartiles (Q25, median, Q75) for element concentrations (ng/ml, w.w.) in whole blood of the red-necked nightjar (*Caprimulgus ruficollis*). Lower limit of quantification (LOQ) given together with the proportion of samples <LOQ. N = 48.

Element	*Group*[a]	*Range (min – max)*	*Q25*	*Median*	*Q75*	*LOQ*	*%<LOQ*
Aluminum (Al)	2	<LOQ - 1300	11.3	23.8	29.6	38.4	88
Antimony (Sb)	2	<LOQ - 3.22	0.010	0.010	0.010	0.010	88
Arsenic (As)	2	0.250 - 58.3	0.800	2.26	6.82	0.008	0
Barium (Ba)	2	<LOQ - 235	16.9	25.9	37.1	1.02	4
Beryllium (Be)	2	<LOQ - 0.356	0.020	0.039	0.045	0.050	81
Bismuth (Bi)	4	<LOQ - 0.233	0.006	0.007	0.008	0.050	98
Cadmium (Cd)	2	<LOQ - 5.35	0.235	0.450	1.46	0.010	10
Cerium (Ce)	3	<LOQ - 4.84	0.608	0.873	1.38	0.050	6
Chromium (Cr)	1	<LOQ - 14.1	1.06	1.71	3.78	0.229	6
Cobalt (Co)	1	<LOQ - 10.1	0.702	1.10	2.53	0.011	4
Copper (Cu)	1	152 - 477	284	352	395	1.72	0
Dysprosium (Dy)	3	<LOQ - 0.064	0.008	0.010	0.023	0.005	52
Erbium (Er)	3	N/A	<LOQ	<LOQ	<LOQ	0.050	100
Europium (Eu)	3	<LOQ - 0.021	0.007	0.008	0.010	0.005	46
Gadolinium (Gd)	3	<LOQ - 0.119	0.009	0.026	0.037	0.005	29
Gallium (Ga)	4	<LOQ - 0.917	0.167	0.232	0.296	0.050	6
Gold (Au)	4	<LOQ - 0.756	0.171	0.414	0.523	0.005	69
Holmium (Ho)	3	<LOQ - 0.010	0.006	0.007	0.008	0.005	79
Indium (In)	4	<LOQ - 0.028	0.007	0.007	0.011	0.005	69
Iron (Fe)	1	80800 - 657000	422000	470000	522000	24.6	0
Lanthanum (La)	3	<LOQ - 4.99	0.040	0.088	0.232	0.100	56
Lead (Pb)	2	<LOQ - 1320	10.1	16.9	182	0.361	4
Lutetium (Lu)	3	<LOQ - 0.010	0.006	0.007	0.009	0.005	96
Manganese (Mn)	1	5.73 - 327	13.5	18.5	32.8	0.371	0
Mercury (Hg)	2	5.83 - 351	15.6	26.0	38.3	0.010	0
Molybdenum (Mo)	1	11.7 - 44.1	24.6	30.5	37.2	0.148	0
Neodymium (Nd)	3	<LOQ - 0.471	0.007	0.009	0.190	0.005	58
Nickel (Ni)	1	<LOQ - 206	2.46	4.52	6.43	7.95	94
Niobium (Nb)	4	<LOQ - 0.317	0.008	0.065	0.097	0.005	38
Osmium (Os)	4	N/A	<LOQ	<LOQ	<LOQ	0.005	100
Palladium (Pd)	2	N/A	<LOQ	<LOQ	<LOQ	0.010	100
Platinum (Pt)	4	<LOQ - 0.010	0.006	0.008	0.009	0.005	98
Praseodymium (Pr)	3	<LOQ - 0.125	0.007	0.008	0.010	0.005	81
Ruthenium (Ru)	4	<LOQ - 0.025	0.007	0.008	0.009	0.005	83
Samarium (Sm)	3	<LOQ - 0.094	0.006	0.008	0.009	0.005	88
Selenium (Se)	1	145 - 1880	694	943	1180	0.153	0
Silver (Ag)	2	<LOQ - 12.8	0.031	0.073	0.088	0.100	79
Strontium (Sr)	2	14.0 - 665	37.4	70.1	116	0.439	0
Tantalum (Ta)	4	<LOQ - 0.104	0.007	0.010	0.014	0.005	10
Terbium (Tb)	3	<LOQ - 0.012	0.006	0.008	0.009	0.005	85
Thallium (Tl)	2	<LOQ - 6.74	0.029	0.041	0.059	0.050	73
Thorium (Th)	2	<LOQ - 0.135	0.024	0.032	0.046	0.050	88
Thulium (Tm)	3	<LOQ - 0.010	0.006	0.007	0.009	0.005	98
Tin (Sn)	2	<LOQ - 4.32	0.010	0.010	0.510	0.010	56
Titanium (Ti)	4	<LOQ - 56.4	14.9	21.5	25.0	0.757	23
Uranium (U)	2	<LOQ - 0.050	0.023	0.027	0.042	0.050	98
Vanadium (V)	2	<LOQ - 6.39	0.027	0.763	1.07	0.050	35
Ytterbium (Yb)	3	<LOQ - 0.031	0.006	0.008	0.010	0.005	48
Yttrium (Y)	3	<LOQ - 0.279	0.009	0.070	0.118	0.005	38
Zinc (Zn)	1	2810 - 19200	4860	5680	7030	51.0	0

[a] Element categories: 1 = Essential trace elements, 2 = ATSDR's list toxic elements, 3 = Rare earth elements, 4 = Other minor elements.

Table 2. Mean (±95% confidence limits) element concentrations (ng/ml, w.w.) in whole blood of the red-necked nightjar (*Caprimulgus ruficollis*) at three sampling environments (agricultural-urban area, mining area, control). Linear models (LM) for comparison of means. Tukey's test: means with the same letter are not statistically different. N = 48.

		Mean		*LM*[a]	
Element	*Agricultural-Urban (CL)*	*Mining (CL)*	*Control (CL)*	$F_{2,42}$	*p*
Arsenic (As)[*]	1.17[a] (0.658-2.09)	6.87[b] (3.56-13.2)	1.56[a] (0.907-2.69)	13.0	**<0.0001**
Barium (Ba)[*]	23.0 (10.9-48.3)	29.8 (12.8-69.2)	17.0 (8.45-34.2)	0.72	0.49
Cadmium (Cd)[*]	0.093[a] (0.042-0.208)	0.609[b] (0.244-1.52)	0.119[a] (0.056-0.254)	7.80	**0.0013**
Cerium (Ce)[*]	0.943 (0.450-1.98)	0.610 (0.263-1.41)	0.625 (0.312-1.25)	0.54	0.58
Chromium (Cr)[*]	1.82 (0.945-3.52)	1.66 (0.789-3.51)	1.45 (0.783-2.70)	0.16	0.86
Cobalt (Co)[*]	0.753 (0.344-1.65)	1.53 (0.627-3.72)	1.33 (0.634-2.77)	1.12	0.34
Copper (Cu)	294 (245-344)	289 (233-345)	323 (277-370)	0.74	0.49
Gallium (Ga)[*]	0.224 (0.147-0.342)	0.179 (0.111-0.289)	0.181 (0.121-0.269)	0.45	0.64
Iron (Fe)	483000 (421000-544000)	434000 (372000-496000)	431000 (377000-485000)	0.67	0.52
Lead (Pb)[*]	7.65[a] (3.63-16.2)	166[b] (71.0-386)	3.87[a] (1.92-7.81)	34.5	**<0.0001**
Manganese (Mn)[*]	13.6[a] (8.15-22.5)	30.5[b] (17.2-54.3)	23.8[ab] (14.8-38.5)	3.28	**0.048**
Mercury (Hg)[*]	15.4 (9.92-23.8)	21.2 (12.9-34.7)	24.8 (16.4-37.4)	1.59	0.22
Molybdenum (Mo)	31.2[ab] (27.0-35.4)	25.1[a] (20.3-29.9)	33.4[b] (29.4-37.4)	5.19	**0.0097**
Selenium (Se)[*]	878 (679-1140)	680 (508-909)	880 (691-1120)	1.64	0.21
Strontium (Sr)[*]	51.1 (33.1-79.1)	62.6 (38.2-103)	63.9 (42.4-96.3)	0.40	0.67
Tantalum (Ta)[*]	0.009 (0.006-0.015)	0.013 (0.008-0.022)	0.009 (0.006-0.015)	0.81	0.45
Zinc (Zn)[*]	5180 (4180-6420)	6020 (4720-7680)	5360 (4380-6560)	0.65	0.53

[*]Geometric means, values $\log_{10}$ transformed for the analyses and back-transformed for the table.

[a] Explanatory factors in the models were study area, age and sex.

Figures

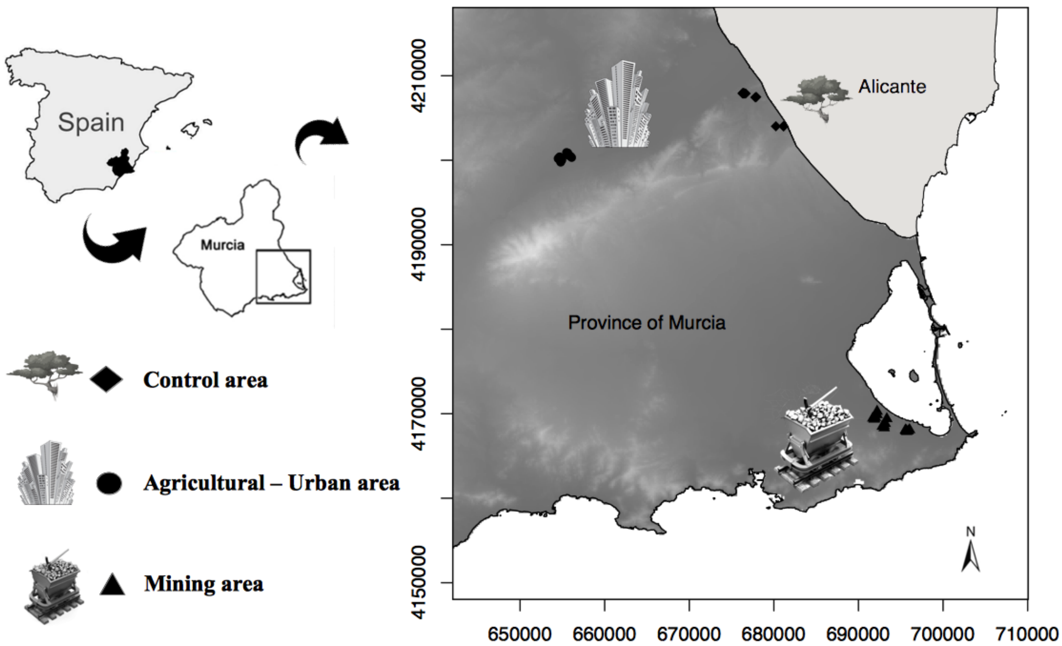

Figure 1. Map showing the geographical location of the studied areas.

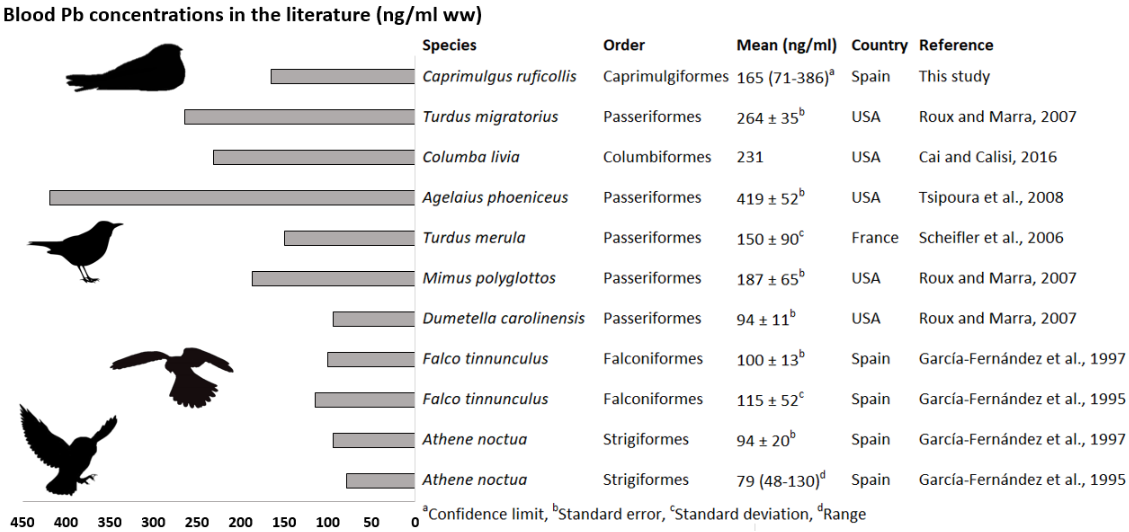

Figure 2. Blood Pb concentrations (ng/ml, w.w.) found in different bird species inhabiting polluted/urban areas in the literature.

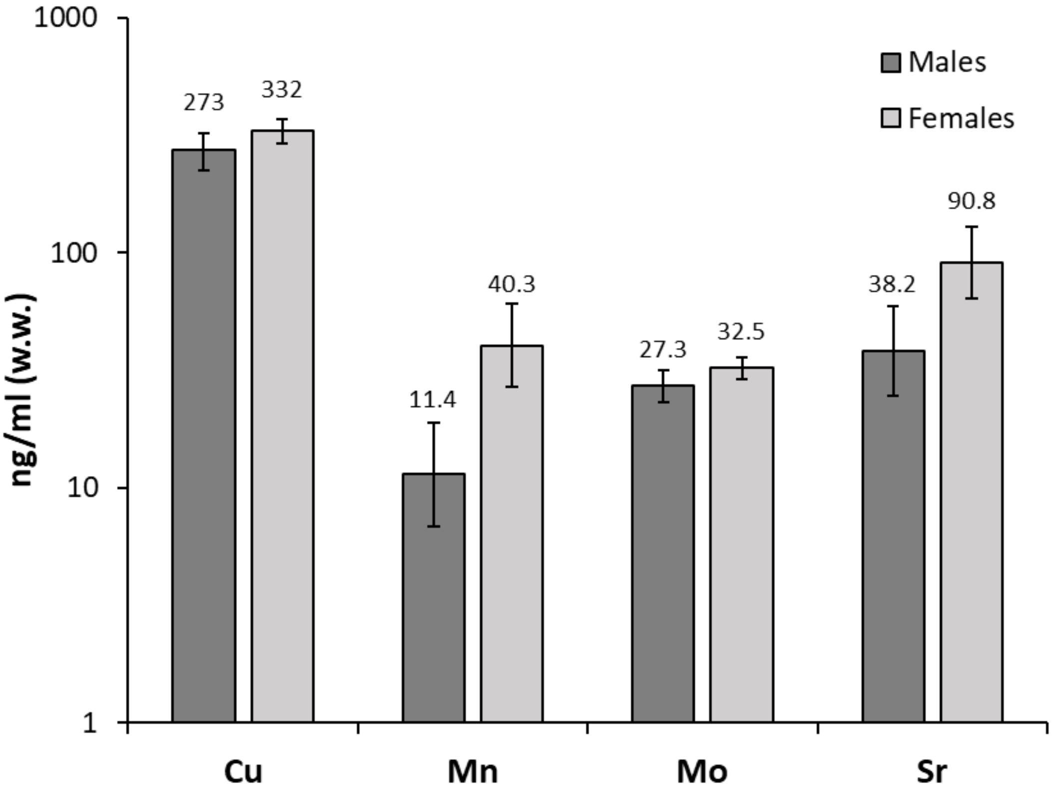

Figure 3. Means (±95% confidence limits) of four elements showing sex-specific differences in blood concentrations of the red-necked nightjar (*Caprimulgus ruficollis*). N = 48 (19 males + 29 females).

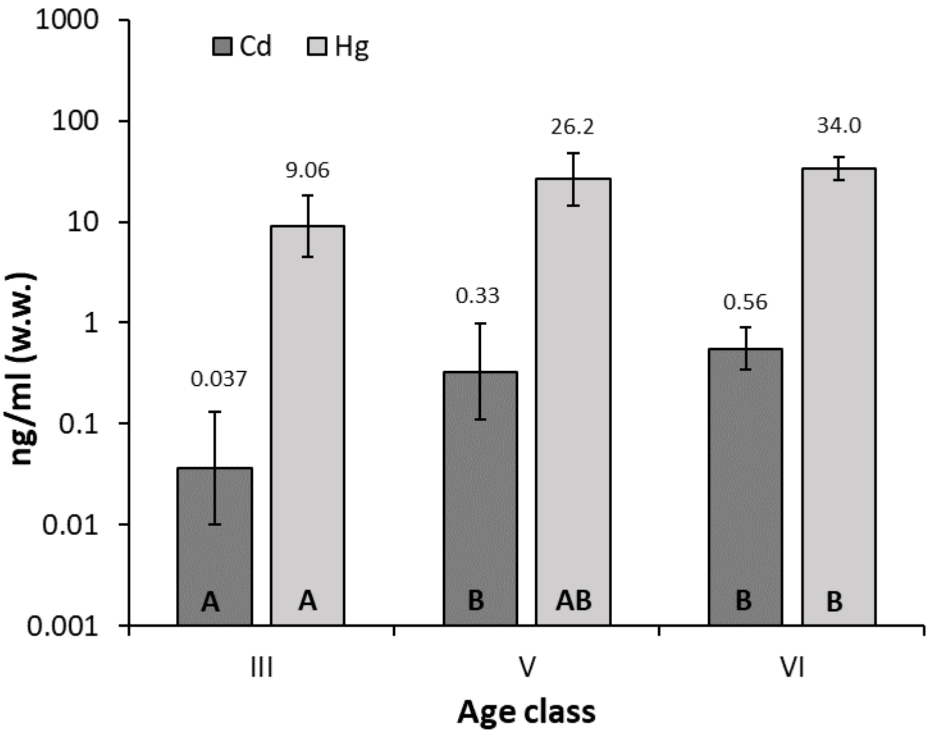

Figure 4. Means (±95% confidence limits) of two elements showing age-specific differences in blood concentrations of the red-necked nightjar (*Caprimulgus ruficollis*). N = 48 (for age classes: III = 5, V = 8, VI = 35). Tukey's test: means with the same letter (within element) are not statistically different.

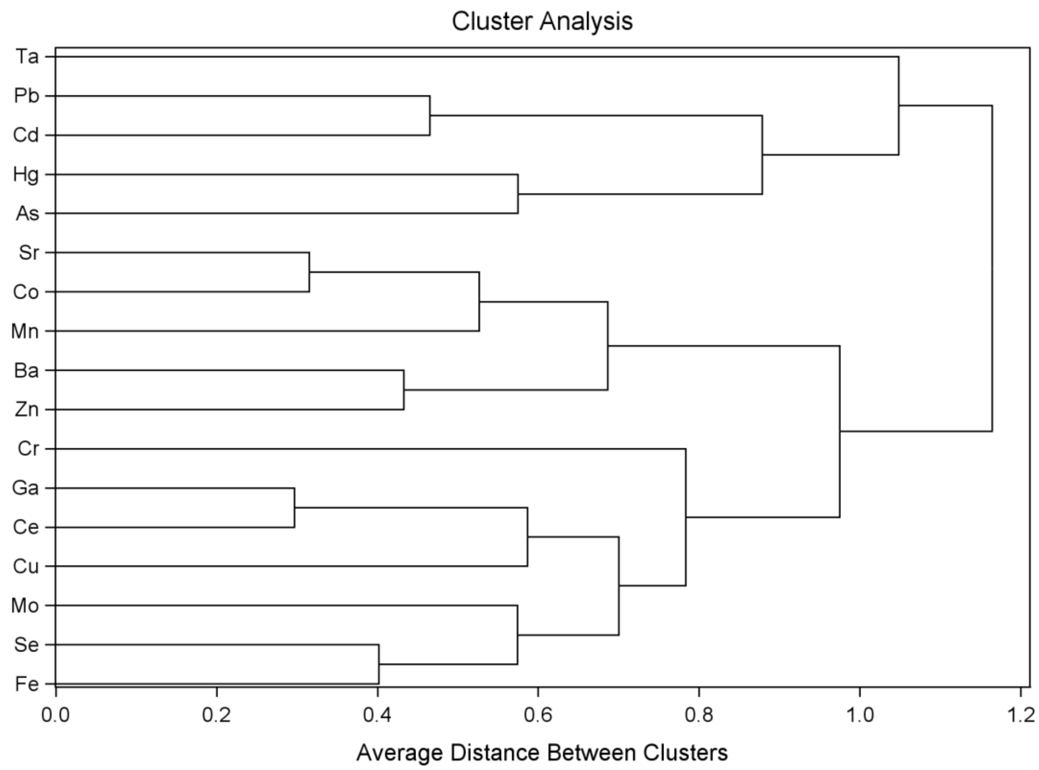

Figure 5. Hierarchical clustering of 17 elements in whole blood of the red-necked nightjar (*Caprimulgus ruficollis*) on the basis of their Pearson correlation matrix. N = 48 for each element.