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Using Pb–Al ratios to discriminate between internal and external deposition of Pb in feathers

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ABSTRACT

Feathers provide a potentially useful biomonitoring option in studies regarding pollution exposure in avian species. However, they must be used with care because the complex, fine structure is highly prone to accumulating surface contamination. This may therefore give a misleading indication of pollutant intake in the animal. Here, data are presented for 4 large scavenging raptor species collected in Spain, and analyses are undertaken on feather barbs and rachis for both Pb and Al concentrations. Aluminium levels are used as a marker of surface contamination by inorganic particulate material. Despite using a thorough washing technique, feather barbs showed significantly higher levels of Pb than did the rachis for all 4 species studied. We also observed a significant correlation ($r=0.782$, $p<0.001$) between Al and Pb levels in the barbs, whilst rachis Al levels were below our detection limit in all samples analysed. Results indicate that the rachis would provide more representative data as regards Pb (or other heavy metal) uptake and tissue deposition within bird tissues during the period of feather growth. As such, data would be more toxicologically relevant.

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1. Introduction

Birds can be lethally exposed to lead (Pb) if they ingest soil contaminated by mining and smelting activities (Beyer et al., 2000), lead paint (Sileo and Fefer, 1987) or lead fishing weights (Birkhead, 1983). However, the most common Pb source associated with clinical poisoning remains, by far, the ingestion of Pb ammunition, which is still widely used for hunting (Fisher et al., 2006; Mateo, 2009).

In order to monitor Pb exposure in wildlife, threshold Pb concentration values for several bird tissues are available, and are now widely accepted (Franson, 1996; Pain, 1996). These values offer guidance and information regarding short term (blood, liver, kidney) and longer term (bone) exposure to Pb. However, such information can only be obtained by the invasive sampling of trapped live birds (blood) or by using hunted birds or those found dead in the field. Such invasive methods may also be prone to cause data bias related to species abundance, behaviour, or habitat use, and they also represent a risk when working with threatened or endangered species. Hence, several non-invasive techniques are now also commonly used to monitor pollutant exposure via the analysis of samples left in the field by birds, i.e., using egg shells (Clark et al., 2009), feathers (Rattner et al., 2008), faeces (Elliott et al., 2008; Yin et al., 2008) and regurgitated pellets (Mateo et al., 2007; Lopes et al., 2010).

Of these, feathers are proposed to be quite good bioindicators of local environmental exposure in nestling (Dauwe et al., 2004) or adult birds (Church et al., 2006). Lead accumulates in feathers through active or passive diffusion from the blood into the feather follicle. Lead deposits into the protein matrix during feather formation, which can take several weeks, and therefore provides a proportional integration of Pb exposure and circulating blood levels for the period of feather growth (Burger, 1993, Rattner et al., 2008; Burger et al., 2009; Finkelstein et al., 2010). Hence, physiologically incorporated Pb in feathers tends to represent reasonably short term exposure and mobilization from internal tissues, and also acts as a potential detoxification or excretion pathway (Burger et al., 2009; Rattner et al., 2008). Metals (including Pb) that are incorporated into feathers during the growth period will also remain in the particular portion of the feather in which it was deposited at any one time point (Burger, 1993). As such, feathers can be sectioned (like hair) in order that temporal trends in exposure can be monitored (Church et al., 2006; Finkelstein et al., 2010). However, one very important consideration when using feathers is that they may be highly prone to surface contamination. Such contamination from atmospheric deposition, preening, and environmental contact (with soil, dust or water) may then give a misleading indication of biologically incorporated contaminant exposure at the time of feather growth (Dauwe et al., 2002; Jaspers et al., 2004). Whilst

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such data may remain indicative of the environmental quality of the habitat in which the bird is residing, it may give a fairly poor indication of actual food chain uptake, ingestion and transfer of pollutants to the animal (Dauwe et al., 2003; Pain et al., 2005). Unfortunately, such surface contamination may also be very difficult to effectively remove using washing techniques in the laboratory.

The analysis of certain metals with very low intestinal absorption rates such as aluminium (Al) or titanium (Beyer et al., 1997, 1999) has been shown to be useful when interpreting Pb levels in faecal excreta, and when trying to identify sources of Pb exposure (such as Pb shot ingestion; Martínez-Haro et al., 2010). Here, we also evaluate whether Al data would be useful when interpreting Pb levels in feathers. Specifically, we compare data derived for Pb and Al in different parts of flight feathers, i.e., in the fine barbs (which may be more prone to surface contamination) and the spine of the feather, the rachis. We hypothesize that a significant association between Pb and Al in feather parts would tend to indicate significant external inorganic particulate contamination. Such contamination limits the feathers usefulness as a non-invasive tool in Pb uptake monitoring. Moreover, we determine whether there is a relationship between Pb levels in different parts of the feather and whether levels correlate with those observed in bones. Like feathers, bones integrate Pb exposure in birds over an extended period of time, and as such, a relationship may be apparent. In providing such data, perhaps certain parts of feathers can be more confidently used in non-invasive monitoring programs regarding Pb exposure in birds, and the potentially misleading data caused by external barb contamination may be avoided.

2. Materials and methods

2.1. Sampling

The outer-most primary wing feathers (8–10th), and the bones (femur) of four bird of prey species (all scavengers) were collected. Samples were from griffon vulture (*Gyps fulvus*, $n=20$), cinereous vulture (*Aegypius monachus*, $n=3$), black kite (*Milvus migrans*, $n=9$) and red kite (*Milvus milvus*, $n=10$). These individuals were found dead in the field, or were found severely injured and died within a few days of admittance to various wildlife rehabilitation centres in Spain.

2.2. Sample preparation for lead analysis

Feather samples were thoroughly washed, first with deionized water, then with acetone, and finally with 2% nitric acid. Whilst in each solution, sonication was used for a 5 min period. The samples were then sonicated and rinsed with deionized water (twice), and then dried in an oven at 36 °C. The barbs were separated from the rachis with scissors. Preliminary cleaning procedure trials revealed the importance of the 2% nitric acid wash, since a pool of feathers analysed without this treatment showed Pb levels 1.5–4 times higher than a comparative pool washed with the acid.

For each barb and rachis sample, 0.2 g was digested using 2.5 ml of 68% nitric acid (HNO_3) in quartz tubes at room temperature for 12 h. Then, 2.5 ml of 30% H_2O_2 were added and the temperature gradually increased to 160 °C (within 1 h), then held at this temperature for 4 h in a block digester (Standard Heatblock, VWR). The digested samples were then diluted to a final volume of 14 ml with deionized water in 15 ml polypropylene (PP) centrifuge tubes.

For bones, all the muscle tissue was removed with a pair of stainless steel scissors and a scalpel, before the femurs were dried to a constant weight. Marrow was removed and 0.5 g of the diaphysis (shaft or mid-section of the femur) was weighed and digested in a microwave oven system (Ethos E, Milestone) using 4 ml of deionized water, 3 ml of 68% HNO_3 and 1 ml of 30% H_2O_2. The digested samples were diluted to a final volume of 50 ml in PP centrifuge tubes with deionized water. Blanks were processed within each batch of digestions of feathers and bones.

2.3. Lead and aluminium analysis

Lead levels were analysed using a graphite furnace-atomic absorption spectroscopy system (AAnalyst 800, Perkin Elmer) equipped with an autosampler (AS800, Perkin Elmer), using 50 μg of $NH_4H_2PO_4$ and 3 μg of $Mg(NO_3)_2$ as matrix modifiers in each atomization. Aluminium was analysed using a nitrous oxide–acetylene flame atomic absorption spectroscopy system (AAnalyst800 equipped with an AS 90 plus autosampler, and a 5 cm N040-0100 burner head; Perkin Elmer). All concentrations given here are expressed as μg/g (ppm) dry weight (d.w.).

Calibration standards were prepared from commercial stock solutions containing 1 g/l of Pb and 1 g/l of Al (Panreac). The limits of detection (LODs, back-calculated to concentrations in samples) for Pb were 0.127 μg/g in feather and 0.054 μg/g in bone, and for Al were 140 μg/g (in feather). Samples which contained levels below the LOD were assigned values of half the LOD for statistical purposes. A reference sample of bone ash (SRM 1400, National Institute of Standards and Technology) was analysed ($n=12$) and the recovery (mean% recovery ± RSE) was 94.5 ± 1.8% for Pb. Bovine liver reference material (BCR185R, Community of Bureau of Reference) was also analysed ($n=8$) and the Pb recovery was 94.4 ± 5.8%. Since Al concentrations were not certified in these CRM materials, another plant based reference material (bush, branches and leaves, NCS DC 73349) was analysed ($n=6$) and the recovery for Al was 100.4 ± 1.69%.

2.4. Statistical analysis

Data for Al and Pb levels in samples were log-transformed to approach a normal distribution. The normality of the data was then tested using the Shapiro–Wilks test, and the homogeneity of variance with the Levene test. In most cases, the data passed the normality and homogeneity of variance tests, at which point parametric tests were used. These were used to analyse the data for differences among species and between different parts (barb and rachis) of the feathers (using one-way ANOVAs with post-hoc Tukey tests). However, since the Pb levels in the rachis and the Al levels in the barbs did not pass the normality and/or homogeneity of variance tests, non-parametric tests were also used (Kruskal–Wallis and Mann–Whitney tests). The relationship between Pb and Al in the various samples (barb, rachis and bone) was studied using Pearson linear correlations. The differences in Pb levels among these samples were evaluated with Wilcoxon paired tests. The relationship between Pb and Al in barbs was also studied with an analysis of covariance, whilst including species as a factor. Since the level of Al in barbs may reflect external feather contamination, the relationship between Pb in the rachis and the barbs was studied using a partial correlation, with Al level in barbs as a control variable.

3. Results

Significant differences were detected among species for Pb concentrations in the rachis ($\chi^2_3=11.04$, $p=0.012$), barb ($F_{3,\ 38}=6.03$, $p=0.002$), and femur ($F_{3,\ 38}=3.62$, $p=0.022$). Griffon vultures contained greater concentrations of Pb compared to red kites (Table 1). This difference was also observed in relation to Al levels in the barb samples ($\chi^2_3=10.69$, $p=0.014$), where griffon vultures again had higher levels than red kites (Table 1). Aluminium in barbs was also detectable in 33–85% of samples (depending on species), whereas all rachis samples were below LOD. The concentration of Pb in barbs was closely correlated with Al levels in the same sample type ($r=0.782$, $p<0.001$; Fig. 1a). This close relationship was also observed when we included species as a factor in an analysis of covariance ($F_{1,\ 37}=43.14$, $p<0.001$). In contrast, Pb in rachis was not correlated with Al in the barb samples ($r=0.174$, $p=0.271$; Fig. 1b).

The mean level of Pb in the barb samples was significantly greater than in the rachis ($Z=5.47$, $p<0.001$), whilst the level of Pb in the bone was greater than in both rachis and barb ($Z=5.63$, $p<0.001$ and $Z=5.08$, $p<0.001$; respectively; Table 1). Rachis Pb was correlated with barb Pb ($r=0.444$, $p=0.003$; Fig. 1c) and femur Pb ($r=0.481$, $p=0.001$; Fig. 2a), but a slightly higher correlation was observed between barbs and femur samples ($r=0.505$, $p=0.001$; Fig. 2b). The correlation between Pb in rachis and barbs improved slightly when Al was included as a control variable in the partial correlation ($r=0.502$, $p=0.001$).

4. Discussion

Here, different parts of the feather (rachis or barb) showed very different Pb levels. The key question is which is actually more representative of biological Pb uptake in the bird? Logically,

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