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Mercury in Feathers of Nestling Eagle Owls, *Bubo bubo* L., and Muscle of their Main Prey Species in Toledo Province, Central Spain

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Mercury is a non-essential trace element which shows high toxicity (Boening 2000). Levels of mercury in the environment are rising and it has been recorded even in the least polluted areas on Earth like the Antarctic ecosystems (Sánchez-Hernández 2000). Bird feathers have been extensively used for mercury pollution biomonitoring (Burger 1994). Mercury levels in feathers reflect the blood levels at the time of its development. Once the mercury is sequestered into the feather structure it becomes highly stable and then only exogenous deposition can increase its levels (Dauwe et al. 2003). The growing feathers are the main excretion way for the mercury body burdens in birds, a fact which leads to higher levels than in other bird tissues. In this way, using feathers allows one to obtain samples from live birds without risk and to document higher mercury concentrations than using other tissues (Burger 1994).

Published reports of mercury levels are available for a wide variety of bird species in several regions of the world (Burger 1994). However, levels of mercury in birds inhabiting terrestrial Mediterranean ecosystems are still poorly documented, with no data available for central Spain. The main aim of the present work is filling this gap, reporting data on mercury levels in feathers of eagle owl (*Bubo bubo*) chicks and in muscle samples from corpses of its main prey, the wild rabbit (*Oryctolagus cuniculus*), collected at the same nests where chicks were growing. The advantages of using feathers of eagle owl chicks are: 1. Chicks have been much less exposed to sources of external contamination than adult birds due mostly to their shorter age, so that mercury content of chick feathers probably reflects mostly physiological deposition (Dauwe et al. 2003; Golden et al. 2003); 2. Levels of pollutants in chicks are likely to represent local exposure in a narrow area around the nest, since parents forage close to nest sites along the breeding season (Ortego and Díaz 2004); and 3. The eagle owl is a top predator in Mediterranean terrestrial food webs, preying on a wide variety of species apart from mammals (Serrano 2000). Given that our study area could be considered scarcely polluted, the obtained data may serve as a reference for other studies and to assess temporal trends in the levels of this pollutant in face of future land-use changes. The density and reproductive output of the studied population is one of the highest reported to date (Ortego 2004; Ortego and Díaz 2004). Establishing the contaminant profiles for these healthy populations of top predators will be useful as reference values for other populations of conservation concern (Straalen and Ernst 1991).

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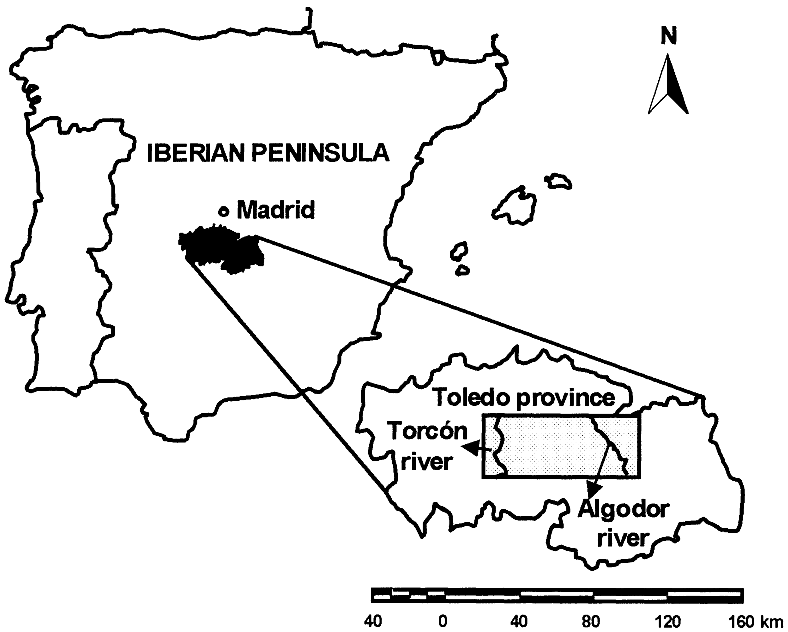

Figure 1. Map of Iberian Peninsula with the location of the study area within the Toledo province.

MATERIAL AND METHODS

The study area extends over 2100 km^2 of the province of Toledo, central Spain (centred on 39°47′ N, 4°04′ W; Figure 1). In March-May of 2002 and 2003, a pinch of feathers was plucked from the breast of all the chicks (20-30 days old) present in the studied nests and were stored in metal-free paper envelopes. We used body feathers since they are more representative of the metal concentrations in the entire plumage than other feather tracts and their sampling does not affect the future chick flight performance (Burger and Gochfeld 1992). For a subset of the studied nests in 2003, a piece of thigh muscle of wild rabbits found dead in the visited nests was collected in a plastic eppendorf tube (adult owls usually stock nests with prey apart from feeding chicks; Ortego 2004). Only very recent rabbit corpses (2-4 h after capture, judging by their appearance) were sampled. Muscle samples were kept on crushed ice until taken to the laboratory within the same day, where both muscle and feather samples were immediately frozen at -80°C until analysis.

Feathers from all chicks belonging to the same nest were pooled to reach enough sample amounts. Then they were washed vigorously in deionised water alternated with acetone to remove external contamination (Gochfeld et al. 1996). This procedure was repeated three times and then samples were put in metal-free vials and dried in an oven at 50°C for 18 hours. Dry feathers were then placed in a dessicator for 2 hours to equilibrate with ambient room temperature (*ca.* 25°C). Feather shafts containing blood were removed (Caldwell et al. 1999) and about 0.1-0.2 g of feather sample were weighed. On the other hand, about 0.2 g of wild rabbit fresh muscle were also weighed. Both tissues were digested in a closed vessel microwave-assisted extraction system (Milestone, Ethos Plus) using 8.0 ml HNO_3 (65% w/v, Merck) and 2.0 ml H_2O_2 (30% w/v, Merck) following an extraction temperature program with five steps (Step 1: 25-200°C, 5 min.; Step 2: 200°C, 5 min.; Step 3: 200-220°C, 5 min.; Step 4: 220°C, 5 min.; Step 5: 220°-25°C, 15 min.). The obtained solutions were each transferred to a 50 ml volumetric flask and the adjusted volumes were stored in well-cleaned polyethylene vials at 4°C until the analyses. The total mercury concentration of the samples was determined by cold-vapour atomic fluorescence spectrometry using an atomic fluorescence detection system (Millenium Merlin 10.025, P.S. Analytical Ltd.) and $SnCl_2$ (4% (w/v) in a HCl 3 mol L^{-1} medium) as the reducing agent. High purity acids and double distilled water were used for the analyses. The accuracy of the method was previously validated with certified reference biological materials (GBW08571 (mussel tissue), TORT-1 (lobster hepatopancreas), DOLT-3 (dogfish liver) and DORM-2 (dogfish muscle): 0.067±0.008 mg g^{-1}, 0.458 mg g^{-1}, 3.37±0.14 mg g^{-1} and 4.64±0.26 mg g^{-1} of Hg, respectively). No statistically significant differences were found between the certified values and the experimental ones at 95 % confidence levels. The percentage of recovery was calculated by processing identical samples which had been spiked with known amounts of the analytical mercury standard (mercury (II) chloride, Panreac, Barcelona, Spain). The mean for recoveries was 107.8 % (S.D.= 8.8; Range= 96.1-117.5; n= 6). Mean relative standard deviations among triplicate measurements with the same sample were bellow 10 %. The proposed method provides a limit of detection for mercury of 4 ng g^{-1}, calculated as three times the standard deviation of ten blank measurements. We determined the water content in muscle tissues by drying samples in an oven at 95°C for 16 hours. All mercury concentrations are expressed on a dry weight basis.

Statistical analyses were carried out in order to determine mercury level differences between nests located in different locations (valleys) of the study area by means of a one-way ANOVA. Differences in mercury levels between rabbit muscle and feathers within the same nest were compared using a Wilcoxon matched pair test. The relationship between the mercury content in chick feathers and rabbit muscle from the same nests was analyzed by means of a nonparametric Spearman rank correlation. All analyses were performed with the STATISTICA software (StatSoft 1996) with a significance level of 5 %.

RESULTS AND DISCUSSION

Mercury levels were analyzed in breast feathers of chicks from 27 eagle owl nests in 2003 and from 5 nests in 2002 (Table 1). Mercury concentrations ranged in both years between 0.024 and 0.217 μg g^{-1} (d.w.; Table 1). Age-related differences in trace element levels are known for birds (e.g., Burger and Gochfeld 2000) and different feather types usually show very variable mercury concentrations as a consequence of the moult sequence (e.g., Dauwe et al. 2003). For these reasons, we should only compare our results with reports for the same type of feathers and bird's age class.

However, no comparable data has been yet reported for terrestrial habitats and data used for comparison (summarized in Table 2) come from bird species linked to marine or freshwater habitats. The concentrations measured in eagle owls can be considered very low as compared with the published reports available (Table 2). Terrestrial food chains are shorter than the aquatic ones, a fact that reduces the importance of biomagnification processes (Palma et al. 2005). This could partially account for the low mercury levels found in eagle owl feathers. Furthermore, no smelting or mining operations, waste incinerators or important industries are present in our study area. In

Table 1. Total concentration of mercury in feathers of eagle owl chicks (μg g^{-1}, d.w.) during 2002 and 2003 and in muscle of rabbits (μg g^{-1}, d.w.) found in the same nests during 2003.

Nest	Chick feathers		Rabbit muscle	Feathers/ muscle ratio
	2002	2003	2003	2003
1	0.04	-	-	-
2	0.08	-	-	-
3	0.09	-	-	-
4	0.19	-	-	-
5	0.22	-	-	-
6	-	0.06		-
7	-	0.05	0.01	4.18
8	-	0.07	-	-
9	-	0.05	-	-
10	-	0.14	0.01	23.17
11	-	0.10	-	-
12	-	0.02	-	-
13	-	0.04	0.01	3.23
14	-	0.12	<LOD	-
15	-	0.04	0.01	2.86
16	-	0.07	0.01	6.27
17	-	0.09	-	-
18	-	0.09	-	-
19	-	0.05	-	-
20	-	0.09	<LOD	-
21	-	0.10	-	-
22	-	0.09	<LOD	-
23	-	0.07	0.01	8.62
24	-	0.05	<LOD	-
25	-	0.02	-	-
26	-	0.12	-	-
27	-	0.16	0.01	12.23
28	-	0.14	<LOD	-
29	-	0.13	0.05	2.78
30	-	0.15	<LOD	-
31	-	0.15	-	-
32	-	0.18	<LOD	-
Mean ± S.D.	0.12 ± 0.08	0.09 ± 0.04	0.01 ± 0.01	7.92 ± 6.99
Range	0.04-0.22	0.02-0.18	LOD-0.05	2.78-23.17

LOD: Limit of detection

this scenario, most mercury inputs are expected to come from fossil fuel burning, long-distance air pollution (Nybø et al. 1996; Rytuba 2003) and natural levels derived from geological processes (Boening 2000; Kruuk et al. 1997). Seeds coated with mercury-based fungicides could have been an additional source in the past, although they have not been sowed at least during the last 20 years (C. Drudis, *personal communication*). In any case, the obtained mercury concentrations are below the levels considered toxic for wild birds in the literature, so that they are not likely to produce any toxic hazard to the studied eagle owl population (reviewed in Palma et al. 2005).

In Toledo province the eagle owl nests are mainly located in cliffs along watercourses (Ortego and Díaz 2004). We studied differences in the mercury levels between the

Table 2. Total concentrations of mercury ($\mu g\ g^{-1}$, d.w.) in contour feathers from chicks of different bird species.

Specie	Mean ± S.E. (range)	N	Area	Ref
Haliaeetus leucocephalus	9.00	115	U.S.A. / Great Lakes	Bowerman et al. 1994
Sterna hirundo	2.61 ± 0.26	21	U.S.A. / New York	Burger and Gochfeld 1992
Sterna hirundo	3.10 ± 0.00	21	U.S.A. / Massachusetts	Burger et al. 1994
Ardeola bacchus	2.40 ± 0.70	5	China / Szechuan	Burger and Gochfeld 1993
Nycticorax nycticorax	2.30 ± 0.47	10		
Nycticorax nycticorax	0.84 ± 0.04	6	Hong Kong	
Bubulcus ibis	1.30 ± 2.80	9		
Egretta garzetta	2.20 ± 0.88	7		
Egretta alba	0.27 ± 0.03	8		
Sterna hirundo	1.40 ± 0.60 [1] (0.6-2.6)	16	U.S.A. / New York	Gochfeld 1980
Diomedea nigripes	5.57 ± 0.36	17	Mid Pacific	Burger and Gochfeld 2000
Diomedea immutabilis	2.15 ± 0.12	35		
Larus argentatus	1.80 ± 0.11	20	U.S.A. / New York	Burger 1995
Gygis alba	1.65 ± 0.11	7	North Pacific	Burger et al. 2001
Sterna fuscata	0.16 ± 0.02	16		
Egretta garzetta	0.21 [2] (0.16-0.25)	3	Pakistan / Haleji lake	Boncompagni et al. 2003
Egretta intermedia	0.16 [2] (0.14-0.22)	3		
Egretta garzetta	0.89 [2] (0.43-2.65)	11	Pakistan / Karachi	
Egretta garzetta	0.97 [2] (0.71-1.32)	2	Pakistan / Taunsa	
Bubulcus ibis	0.41 [2] (0.02-1.48)	10		
Pterodroma hypoleuca	3.87 ± 0.32	20	North Pacific	Gochfeld et al. 1999
Peaton rubricauda	2.51 ± 0.28	12		
Puffinus nativitatis	0.34 ± 0.00	9		
Nycticorax nycticorax	35,25 [2] (21.93-67.07)	16	U.S.A. / Nevada (mining)	Henny et al. 2002
Egretta thula	30.64 [2] (14.68-59.77)	14		
Phalacrocorax auritus	66.31 [2] (54.0-87.3)	6		
Nycticorax nycticorax	5.51 [2] (3.42-16.56)	15	U.S.A. / Nevada (reference)	
Egretta thula	7.77 [2] (5.65-12.24)	14		
Phalacrocorax auritus	11.81 [2] (10.9-13.5)	3		

[1] S. D.; [2] Geometric mean

nests placed in the Torcón and Algodor rivers, situated in the west and east borders of the study area, respectively (Figure 1). In spite of the low sample sizes, we found marginally significant differences between river catchments ($F_{1,8}$= 4.47; P= 0.067), with the nests located in the Algodor river (mean= 0.057; S.D.= 0.011; Range= 0.046-0.069; n=3) showing lower mercury levels than those placed in the Torcón river (mean= 0.106; S.D.= 0.038; Range= 0.046-0.151; n=7). Given that both valleys do not differ significantly in the presence of anthropogenic sources of mercury and their geology is very similar (Crespo 1993), the observed differences could be mostly attributed to differences in rainfall (Boening 2000; Kruuk et al. 1997). Precipitations are known to be higher in the west of the study area (Crespo 1993), a fact which facilitate mercury wet deposition and could explain the observed marginal spatial variation in its levels (Kruuk et al. 1997; Palma et al. 2005). However, differences in diet between the pairs settled in both valleys can not be ruled out as an influential factor affecting the results (Palma et al. 2005) but, unfortunately, no data are available to test this hypothesis.

Mercury levels found in muscle from seven wild rabbits were under the detection limit and the maximum value recorded was 0.014 $\mu g\ g^{-1}$ (d.w.; Table 1). Wild rabbit muscle had lower mercury concentrations than feathers of eagle owl chicks growing in the same nests (Z= 2.52; P= 0.01; n= 8). Mercury concentrations in feathers were on average 7.9 times higher than in rabbit muscle samples (Table 1). As previously found for Bonelli's eagles in southern Portugal (Palma et al. 2005), biomagnification processes seem to be the most likely cause for these prey-predator differences (Straalen and Ernst 1991). This biomagnification factor was lower than the values reported by Henny et al. (2005) for young American dippers (*Cinclus mexicanus*) feathers (10.3-20.4), a fact probably related with the greater importance of biomagnification processes in aquatic environments. However, we failed to find a relationship between mercury concentrations in feathers and rabbit muscle from the same nests (r_s= -0.24; p= 0.57; n= 8). Although rabbits are the main prey of eagle owls in the studied population (Ortego 2004), owls could be acquiring most of their mercury burdens through the intake of secondary consumers such as carnivores and other raptors (Serrano 2000). These prey, although far less important in terms of ingested biomass (Serrano 2000), are expected to have higher mercury levels due to biomagnification and hence influence owl's mercury profiles more than herbivore prey (Palma et al. 2005).

Our data suggest that breast feathers of eagle owl chicks could be adequate indicators of mercury contamination in terrestrial ecosystems. As a top predator, the eagle owl appears to accumulate mercury from lower trophic levels. However, establishing conclusive relationships between mercury concentrations in eagle owls and their prey requires additional data on mercury levels in other prey species and on the diet composition of the studied pairs (Palma et al. 2005). The extremely low concentrations found in samples of eagle owl prey, sometimes under the detection limit, makes more adequate the use of feathers of top predators as matrix for mercury determination in future research. On the other hand, the terrestrial Mediterranean ecosystems of Toledo province appears to be scarcely polluted, and mercury levels reported here could be suitable reference values for other populations moderately or highly exposed to mercury contamination. However, the absence of comparable data for terrestrial ecosystems and values for contaminated and reference sites makes necessary further research on mercury levels in birds integrated in terrestrial food webs.

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