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Cadmium in Feathers of Adults and Blood of Nestlings of Three Raptor Species from a Nonpolluted Mediterranean Forest, Southeastern Spain

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Cadmium is one of the most toxic heavy metals for living beings. Its environmental ubiquity and persistence, its accumulation in organisms and biomagnification along the trophic chain, imply a continuous exposure to low doses. This metal can be fatal in cases of acute poisoning, or can indirectly affect avian populations by altering reproductive success, behavior, immune response and physiology, and may also induce cancer in cases of chronic exposure (Burger and Gochfeld 2000). Sometimes wild birds may be exposed to very high levels of metals, for example at waste disposal sites (Aznalcóllar, Spain 1998). Acute poisonings are easily diagnosed, although long-term effects are difficult to assess since background levels associated with low or non-exposure are not known for many species.

Cadmium in blood is a good indicator of recent exposure (Garcia-Fernandez et al. 1996), while chronic exposure may be considered when data on concentrations in accumulator tissues (kidney) are available. Although studies on animal corpses offer useful information, ethical, legal and scientific reasons point to the need for other types of more easily available samples (feathers, eggs, excrement, regurgitated food, etc), which allow us to approximate exposure conditions (Burger and Gochfeld 2000, Dauwe et al. 2000). In recent years, the usefulness of feathers as a biomarker for heavy metal exposure has been the subject of research. Results have proven satisfactory for monitoring mercury and lead levels, while contradictory results have been obtained for cadmium due to many factors such as variations in feathers, variations among different parts of a feather, molting patterns, and changes of diet during molting (Furness 1993, Martinez-Lopez et al. 2004).

Chemical and biochemical parameters have been used for many years in both field and experimental studies on wild birds. Experimental studies, in which all factors are strictly controlled, provide important information, although doubt remains concerning whether all the factors used in experimental studies are present in natural conditions. Furthermore, ethical and logistical reasons hinder experimental studies on certain species, especially large raptors, many populations of which are in jeopardy due to habitat limitations and disturbance. In field studies, we have no control over exposure associated with prey and water ingestion. Normally, biomonitoring studies use an uncontaminated area as a reference for comparing

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results obtained in contaminated areas. However, knowledge of the levels of chemical parameters associated with background exposure to cadmium is scarce or null for many species, especially raptors.

This study was carried out on three forest raptor species: the booted eagle (*Hieraaetus pennatus)*, goshawk (*Accipiter gentilis)* and common buzzard (*Buteo buteo*) which inhabit a Mediterranean forest with no specific sources of heavy metal pollution, and can be considered as an uncontaminated, remote or reference zone for field studies. We analyzed cadmium in the blood of raptor nestlings to estimate recent exposure in these three species. Cadmium in primary feathers from parents was used in order to evaluate its utility as an alternative tool for biomonitoring. Finally, another aim of this study was to obtain useful information on cadmium background exposure in these raptor species to serve as a reference for biomonitoring studies on populations either moderately or highly exposed to this metal.

MATERIALS AND METHODS

The study area (38°00' N, 1°46' W) covers about 10,000 ha and ranges from 550 to 1521 m. It consists of a mountainous area of Mediterranean forest, surrounded by dry farming crops and far from any urban, industrial or mining zones. The nearest industrial area, urban area (population > 350.000 inhabitants) and highway are situated 70 km, 60 km and 70 km away, respectively. The forest is composed of *Pinus halepensis*, with an undergrowth of sclerophyllic shrubs (*Quercus coccifera*, *Pistacia lentiscus*, etc.). Typical agricultural crops are grapes, almonds, olives and cereals. The climate is typically Mediterranean with cold winters and warm summers. Forest raptors in the zone are represented by the booted eagle (26 pairs in 1999 and 21 pairs in 2000), the goshawk (1 pair in 1999, 2 pairs in 2000), and the common buzzard (7 pairs in 1999, 6 pairs in 2000). Diet is similar for all three species during the breeding season and includes medium-sized birds (such as ring-doves), rabbits and lizards. Each pair used the same nest both years (Martinez 2002). In southeastern Spain, common buzzard and goshawk populations are sedentary, however, the booted eagle is considered a trans-Saharan migrant (Sánchez-Zapata and Calvo 1999).

During the 1999 and 2000 breeding seasons, the reproductive behavior of the three species (booted eagle, goshawk and common buzzard) was observed from the beginning of courtship (March-April) until the nestlings left the nest (June-July). Close to the predicted date for hatching, the nests were visited twice weekly. In order to obtain a sufficient volume of blood, the nestlings were sampled between 35 and 45 days old. At the same time primary feathers from the mother, if any, were taken. We assume these were feathers from the mother as once the female has eggs, she is dependent on the male for food and spends all her time in the nest (Reading 1990, Martinez 2002) and thus it is more probable the mother's molting feathers were deposited in the nest. One blood sample from nestlings and one feather from the mother were collected per nest.

In order to avoid stressing the nestlings, careful steps were taken. The head of nestling was covered before placing the animal into a bag and bringing it down to the ground where a blood sample was taken by the veterinarian from the "Santa Faz" Wildlife Recovery Center (Alicante, Spain), who also evaluated the health status of the nestlings. Finally, the nestlings were returned to the nest. Blood samples from nestlings (1 ml) were taken by puncturing the radial vein using a hypodermic needle and syringe. These were immediately taken to the laboratory in refrigerated conditions and frozen to –40°C until processing. Feathers found in the nests were collected and kept in bags at room temperature in a cool, dry place. Thirty-four blood samples were collected as follows: 27 from booted eagles (9 in 1999 and 18 in 2000), 4 from common buzzards (all in 2000) and 3 from goshawks (all in 2000). A total of 44 adults' feathers were collected: 30 from booted eagles (20 in 1999 and 10 in 2000), 9 from common buzzards (4 in 1999 and 5 in 2000) and 5 from goshawks (4 in 1999 and 1 in 2000).

Samples were prepared for cadmium analysis via Anodic Stripping Voltammetry (ASV) by eliminating all organic impurities that might interfere with the results. Complete digestion was ensured by using high-temperature digestion with a mixture of acids following the method described by Garcia-Fernandez et al. (1995). All reagents used were Suprapur® quality from Merck (Darmstadt, Germany). The quartz tubes used for wet digestion were previously washed with 2% nitric acid for 48 hrs and then rinsed twice with tetradistilled water and dried in an oven at 100°C. A volume of 0.2 ml of whole blood was placed in a quartz digestion tube, to which 0.5 ml of an acid mixture (nitric/perchloric/sulphuric, 8:8:1) was added. The sample was then submitted to a progressive thermal treatment and, once dried, was left to cool. Tetradistilled purified water was added and transferred to the measuring vessel, adjusting the final volume to 10 ml.

Whole primary feathers were alternately washed in acetone and Triton X-100 diluted to 1:400 (Hughes et al. 1997) to remove loosely adherent external contamination. They were then cut and dried at 80°C for at least 12 hours. The samples were then placed in LDPE (low-density propylene) flasks with an acid mixture (nitric/perchloric/sulphuric, 8:4:1) until the organic matter had completely disintegrated (Martinez-Lopez et al. 2002). One milliliter of the acid mixture was used per 100 mg of feather. Finally 1 ml of these pre-digested extracts was exposed to a heat treatment similar to that described for the blood samples.

Prior to ASV, 100 µl of hydrochloric acid was added to the measuring vessel as an electrolyte support. The pH of the final solution was between 1 and 2. The Anodic Stripping Voltammeter used (VA-646 processor and VA-647 workstation, Metrohm, Switzerland) was equipped with three standard electrodes: a working electrode (hanging mercury drop), reference electrode (Ag/AgCl, KCl 3 mol/L) and auxiliary electrode (platinum). We used the Differential Normal Pulse technique with an electrolysis time of 180 seconds and modulation amplitude of 50 mV. The cadmium concentration in the digested sample was calculated after twice adding dilutions prepared from standard solutions of cadmium (Sigma, St. Louis, MO). The detection limit was 0.25 µg/L. The recovery, which was

determined by analyzing ten identical samples of reconstituted lyophilized blood (European Union Reference Standards) CRM195, was 94.2 ± 3.2% (Garcia-Fernandez et al. 1995).

Statistical analysis of the data was performed using SPSS v10.0 statistical software (SPSS Inc., 1989-1999). Thirty-three percent of the blood analyzed for cadmium showed non-detected values, which were assigned ½ the detection limit established when mean comparison tests were performed. Cadmium concentrations recorded for both sampling years (1999 and 2000) were compared for each species. Given the scarcity of available data for the common buzzard and goshawk, it was impossible to tell whether data were distributed normally. Therefore, comparisons were made by means of the non-parametric Mann-Whitney test. Comparisons of blood-cadmium concentrations could only be made for the booted eagle, whereas those for concentrations in feathers were able to be performed for all three species. The level of significance was set at α=0.05. No significant differences were found between 1999 and 2000 and so the data for both years were pooled in the statistical studies (Table 1).

Table 1. Contrast statistics for cadmium concentrations.

	Blood	Feathers		
	Booted eagle (*H. pennatus*)	Booted eagle (*H. pennatus*)	Common buzzard (*B. buteo*)	Goshawk (*A. gentilis*)
Mann-Whitney's U*	60.50	92.00	0.00	5.00
Wilcoxon's W	105.50	147.00	10.00	15.00
Kolmogorov-Smirnov's Z	-1.104	-0.352	-1.414	-1.225
Probability	0.298	0.746	0.400	0.286

*Mann-Whitney test using an SPSS 10.0 statistical package for mean comparisons between sampling years (1999 and 2000).

Since the sample size was very small for the common buzzard and goshawk, the non-parametric Kruskal-Wallis test was used in order to detect differences between species. The level of significance was set at α=0.05. When statistical differences were found between the three species studied, the Mann-Whitney test for each pair of species was used. Spearman's non-parametric rank correlation test was applied to examine any relationships between mother feathers and chick blood as regards its cadmium content.

RESULTS AND DISCUSSION

Cadmium levels in the blood of nestlings and the feathers of their mothers in three raptor species sampled from an unpolluted forest in southeastern Spain have been examined as tools for biomonitoring exposure to this metal. Cadmium in the blood of nestlings probably reflects their diet, the prey from the forest in which they were born. On the other hand, cadmium in the feathers of their parents reflects the history of the feathers, when and where the feathers grew, subject to

changes over time. During the formation of feather the vascularization, which provides nourishment for cells, produces direct contact between feathers and the rest of the organism, so that metals may reach them via the blood (Dauwe et al, 2000). Once the feather is completely formed, the vascular connection atrophies leaving the feather as a record of blood metal concentrations accumulated during its formation (Spahn and Sherry, 1999).

Information on seasonal migration or residential periods of the three study populations is available (Sanchez-Zapata and Calvo 1999). In this area, the common buzzard and goshawk populations are sedentary and therefore cadmium deposited in feathers reflects the exposure conditions of the zone. However, the booted eagle is considered a trans-Saharan migrant, which overwinters in Africa from mid-October to mid-March (Martinez 2002). We have no relevant information about the molting pattern of the booted eagle, and available information on the other species is scarce. Primary feathers from female goshawks (Reading 1990), common buzzards (Ontiveros 1995), and booted eagles (Forsman 1999) are molted annually. For goshawks, the start of the annual molt occurs between day 134 and 145 (from mid to late May) and ends between late July and September with all primaries replaced every year (Reading 1990). On the other hand, the mean growth rate for primary feathers in the common buzzard from Granada (southeastern Spain) was 4.6 mm/day (with a maximum value of 10 mm/day) and was associated directly with the final length of the feathers, the longest feathers displaying the highest growth rates (Ontiveros 1995). Taking into account all these aspects, every primary feather should be completely formed in less than 90 days. Since we collected primary feathers deposited in the nest during the incubation and breeding season (June) it is highly probable that new feathers are completely formed several days or weeks before migratory flight towards Africa (mid-October) and therefore the cadmium accumulated in these feathers could be related to the exposure conditions of the study area.

Cadmium concentrations in blood (Table 2) were generally low, ranging from undetected in some cases to 1.5 μg/dl, with no statistical differences between species. There are no established background cadmium levels for birds, while concentrations in humans not exposed to cadmium in the workplace are below 0.5 μg/dl (Bernard and Lauwerys 1984), a concentration accepted by the American Conference of Governmental Industrial Hygienists as normal (ACGIH 1994). Data for raptor species are scarce, but cadmium concentrations of up to 0.6 μg/dl with a mean of 0.35 have been found in the nestlings of great horned owls (*Bubo virginianus*), while in the nestlings of western screech owls (*Otus kennicotti*) the mean value (1.7 μg/dl) was ten times higher than that found in our study, with a maximum of 3.2 μg/dl for screech owls (Henny et al. 1994). However, the samples obtained by these authors came from near the Coeur d'Alene River, in northern Idaho and would presumably have been contaminated by the mining activity of Silver Valley. Furthermore, direct comparison with the data from Henny et al. (1994) is difficult since the detection limit of the analytical technique they used was very high (0.5 μg/dl) and 91% of our samples had a cadmium concentration below this value.

Table 2. Cadmium (μg/dl) in blood of nestlings and feathers (μg/kg) of adult raptors sampled in 1999 and 2000 breeding seasons in an uncontaminated forested area of the Region of Murcia (southeastern Spain).

Species	Statistics	Feathers Cadmium (μg/kg)	Blood Cadmium (μg/dl)
Booted eagle	Mean ± S.D.	18.0 ± 8.54	0.30 ± 0.30
	Min-max (n)	5.79-38.2 (30)	n.d. (10)-1.29 (27)
Common buzzard	Mean ± S.D.	28.1 ± 14.7	0.16 ± 0.07
	Min-max (n)	4.27-58.5 (9)	n.d.(3)-0.27 (4)
Goshawk	Mean ± S.D.	18.0 ± 7.22	0.58 ± 0.79
	Min-max (n)	7.52-26.5 (5)	n.d. (2)-1.50 (3)

n.d.(n) = non-detected (number of samples)

Scientific literature on cadmium in raptor feathers is scarce (Movalli 2000), and much less extensive than that on seabirds (Burger 1993, Burger and Gochfeld 1996) and other species (Burger and Gochfeld 1996, Fasola et al. 1998). Since the study area in question is rural and free of large urban, mining and industrial zones, it was to be expected that the levels of cadmium in feathers would be low and could serve as a reference in environmental biomonitoring studies for monitoring the environmental state of the region.

The results published suggest that feathers are not useful as biomonitors for cadmium exposure monitoring. However, Burger and Gochfeld (2000) found substantial levels of cadmium in the feathers of several seabird species (47.1-573 μg/kg). In a review of studies on cadmium in the feathers of several species, Burger (1993) calculated a median of 100 μg/kg for cadmium. The highest values in our study were 58.5 μg/kg for cadmium in a common buzzard (Table 2) and no inter-species differences were found in feathers (χ^2=5.499; p=0.064). In no case were concentrations close to those cited in literature as causing any kind of effect. For example, Burger and Gochfeld (2000) cited values of 2 mg/kg of cadmium as being related with several behavioral, physiological and nutritional disorders in birds. We agree with Burger and Gochfeld (2000) that there is a need for data on the metal content of the feathers of different bird species exposed to different levels of pollution and that each approach provides useful information.

An important aim of this study was to survey the relationship between cadmium concentrations in the feathers of adult raptors and those in the blood of their corresponding nestlings (Figure 1) in order to assess the usefulness of feathers as biomonitors of environmental exposure to these metals. Usually, studies correlate metal concentrations between blood and feather concentrations sampled from the same individuals; however, we have found no references in literature using different samples of nestlings and their parents. However, this relationship can only be used for sedentary species and for those migratory species with an annual molt that completes the formation of primary feathers before the start of migratory flight. In this study, positive relationships (rho=0.869) allow us to suggest that feather samples from these raptor species are able to offer time-specific information on their state of health, at least in uncontaminated forest areas.

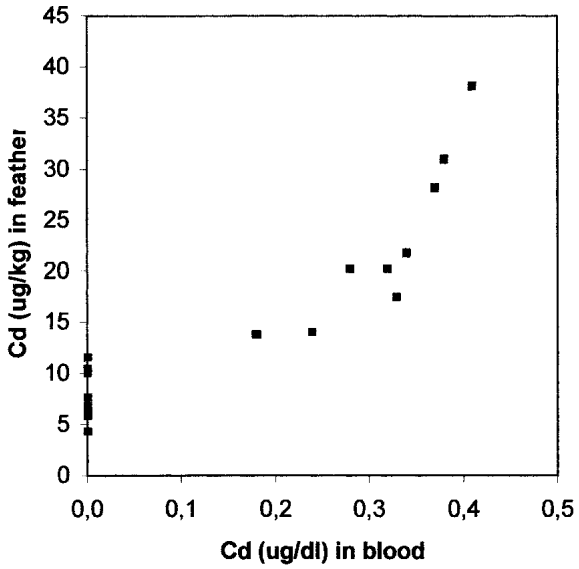

Figure 1. Relationships for cadmium concentrations in blood and feathers (rho=0.869, $p<0.01$).

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