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# Use of Raptors for Biomonitoring of Heavy Metals: Gender, Age and Tissue Selection

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**Abstract** The concentrations of cadmium, mercury and lead were determined in liver, kidney and feathers of *Accipiter gentilis*, *Buteo buteo* and *Strix aluco*. No significant differences in the concentrations of metals were observed in relation to either age or gender. Cadmium levels in feathers and in kidney were significantly correlated ($p < 0.01$ or $p < 0.05$) in all species. In *B. buteo*, cadmium concentrations in feathers and in liver were also correlated ($p < 0.01$), as were the concentrations of lead in feathers and in liver ($p < 0.05$). The low correlation coefficients let to reject the possibility of predicting metals concentrations in viscera from the concentrations in feathers.

**Keywords** Viscera · Feathers · Pollution · Bioaccumulation

The use of birds as biomonitors provides a powerful tool to study the environmental quality of ecosystems (Furness 1993). Although it has been paid attention to the study of metal content of seabird organs (Gochfeld et al. 1996; Monteiro et al. 1998; Burger and Gochfeld 1999), little is known about the accumulation of heavy metals in terrestrial birds. Anthropogenic influences are greater in terrestrial environments as a result of many different industrial and urban activities, which are often sources of heavy metal pollution. In this context, birds of prey are ideal biomonitors because they at the top of the food chains, have a wide geographical distribution and are sometimes sedentary.

The concentrations of metals in internal tissues represent the levels of metals in the diet (Lewis and Furness 1991, Monteiro et al. 1998), which in turn reflect the degree of contamination of the ecosystem. However, there are certain drawbacks associated with the analysis of internal organs, such as seasonal variations in weight and the need in some cases to sacrifice a large number of birds to obtain sufficient samples (Furness 1993). Feathers are an alternative source of tissue because they are obtained non-invasively, reflect the metal concentration in the blood at the time of their formation (Goede and de Bruin 1984; Goede 1985) and indicate the amounts derived from the diet.

Metal accumulation varies in relation to certain aspects such as gender or age of the birds. Sometimes these characters are ignored and homogeneous samples are often used to facilitate the analysis, with the consequent risk of error in the results. Although gender and age-related differences have been reported for a large number of species, there is no clear pattern in relation to elements or tissues. The lack of significant differences may be due to an insufficient number of samples or the difficulty in determining the exact age or sex of birds (Gochfeld et al. 1996; Burger and Gochfeld 1999).

Numerous dead birds of prey are stored in the Environmental Specimen Bank of Galicia and they will allow us to obtain consistent results. The most abundant species are *Accipiter gentilis*, *Buteo buteo*, and *Strix aluco*, all previously used in studies of heavy metal accumulation (Battaglia et al. 2005; Martin et al. 2008; Pérez-López et al. 2008). The objectives of this study were: (1) to examine the effects of gender (and when sample sizes allow, the effects of age) on tissue concentrations of metals in the birds studied, and (2) to determine whether the concentrations of

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metals in feathers could be used as an indicator of the concentrations in internal organs of the three species studied, with the aim of using feathers as a target tissue for biomonitoring of the metals considered.

## Materials and Methods

The tissues analysed were taken from the bodies of raptors donated by four Wildlife Recovery Centres in Galicia (NW Spain) between 1997 and 2005. A total of 88 individuals were obtained: 16 specimens of *Accipiter gentilis* L., 1758, 38 of *Buteo buteo* L., 1758 and 34 of *Strix aluco* L., 1758. The species selected are suitable for biomonitoring studies because their food is limited to certain types of prey and because they are usually sedentary. In the specific case of *B. buteo*, there is some migratory movement from northern Europe in spring and autumn and therefore only samples from summer season were analysed. The bodies were stored frozen, at $-30^{\circ}$ C, until being processed. The birds were sexed by internal examination, and classified as juveniles (less than 2 years old) or adults (over 2 years old) on the basis of the plumage and gonad condition. Samples of liver and kidneys were obtained by dissection of the corpses. Primary feathers were removed from the right wing of each specimen; feather n° 2 was used for Cd and Pb analysis and feather n° 6 for Hg analysis. Feathers that had not developed completely, or that were broken or deteriorated were rejected. The feathers were washed with bidistilled water and Triton X100 in an ultrasonic water bath for 15 min, then rinsed in bidistilled water with shaking for 1 min. After this in each feather the barbs were separated from the shaft. All tissues were homogenized and dried at 45°C.

After acid digestion with $HNO_3$ the concentrations of Cd and Pb in viscera and feathers were determined by graphite chamber atomic absorption spectrophotometry (Perkin Elmer Analyst 600). The Hg contents were determined with a mercury elemental analyser (DMA 80 Milestone). To ensure the analytical quality, certified reference materials (CRM) were included in the analyses: pig kidney (BCR 186, Community Bureau of Reference), bovine liver (SRM 1577b, National Institute of Standards and Technology) and human hair (GBW 07601, Institute of Geophysical and Geochemical Exploration). The range of recovery of the CRM ranged between 89 and 128%. Duplicate samples were also analysed for each metal; the overall error was less than 20%, except for Cd and Pb in feathers (24 and 22%, respectively). Except for a few exceptions the values obtained were higher than the limit of quantification for each element: 24.0 and 2.43 ng $g^{-1}$ for Cd (viscera and feathers, respectively); 162 and 136 ng $g^{-1}$ for Pb; and 10.3 and 9.8 ng $g^{-1}$ for Hg.

Non parametric statistical tests were used after it was found that the data were not normally distributed and that they could not be normalized by log transformations. A Mann–Whitney U test was used to test for significant gender- and age-related differences between the concentrations of elements in the tissues. The relation between the metal contents in the different tissues was studied by Spearman *Rho* correlations. The statistical analyses were carried out with SPSS 15.0 software.

## Results and Discussion

The descriptive statistics for bioaccumulation in tissues of *A. gentilis*, *B. buteo* and *S. aluco* are shown in Table 1. Comparison of the concentrations obtained in this study with those obtained in other recent studies with the same species (Battaglia et al. 2005; Martin et al. 2008) reveals considerably higher concentrations in our case. The high accumulation of metals in the birds in Galicia may be due to high levels of contamination by these three elements in the northern part of the study area due to the presence of different sources of pollution. In this way the present results are within the same range as those observed in other study of heavy metals in livers of raptors in the same region (Pérez-López et al. 2008), except for the concentrations of Cd in *B. buteo* and Pb in *A. gentilis*, which were higher in the present study, and those of Cd in *S. aluco*, which were lower. However, unlike in the latter study, in the present study for *B. buteo* we only analysed specimens obtained in the summer, to avoid including birds that had migrated from northern Europe. By other hand, our study also involved the analysis of many more specimens than in all previous cited studies.

Regarding to age, in those cases in which comparisons were carried out ($n \geq 5$ for both compared groups) there were no significant differences in the metal concentrations in adult and juvenile birds and therefore the data were considered together. Our data are consistent with those obtained in previous studies (Wayland et al. 1999; Stout and Trust 2002; Martin et al. 2008), although other studies have reported age-related increases in the concentrations of Cd and Pb in the viscera of birds (e.g. Zaccaroni et al. 2003). Although juvenile specimens would have been exposed for less time to the contaminants, the concentrations in these birds were not significantly lower than in older birds, possibly because of the elimination of large quantities of metals during moulting (Burger 1993; Hughes et al. 1997).

In the same way, gender did not have any effect on the metal concentrations in the tissues of the birds analysed, so they were grouped and treated together for correlation analyses. The lack of any significant differences in the

**Table 1** Descriptive statistics for Cd, Pb and Hg (ng $g^{-1}$ dry weight) in liver (L), kidneys (K) and feathers (F) of *Accipiter gentilis*, *Buteo buteo* and *Strix aluco* from Galicia (NW Spain)

| | | *n* | | Cd | | | Pb | | | Hg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | L | K | F | L | K | F | L | K | F |
| *A. gentilis* | ♂ Mat. | 5 | Median | 146 | 818 | 39.8 | 480 | 909 | 1,545 | 236 | 390 | 647 |
| | | | SD | 145 | 340 | 60.6 | 1,580 | 1,680 | 770 | 758 | 919 | 838 |
| | | | Min. | 57.6 | 350 | 10.5 | 222 | 374 | 606 | 141 | 279 | 485 |
| | | | Max. | 428 | 1,111 | 158 | 3,980 | 4,546 | 2,654 | 1,900 | 1,920 | 2,400 |
| | ♂ Juv. | 6 | Median | 30.6 | 120 | 11.5 | 350 | 303 | 2,084 | 354 | 500 | 329 |
| | | | SD | 24.6 | 73 | 6.30 | 434 | 909 | 21,620 | 700 | 727 | 378 |
| | | | Min. | 23 | 51.5 | 7.30 | 132 | 140 | 465 | 164 | 216 | 77 |
| | | | Max. | 86.9 | 259 | 23.2 | 1,313 | 2,188 | 50,000 | 1,960 | 1,940 | 1,180 |
| | ♀ Mat. | 4 | Median | 154 | 1,560 | 29.6 | 798 | 1,020 | 2,244 | 182 | 118 | 1,120 |
| | | | SD | 75.8 | 9,100 | 17.4 | 388 | 3,100 | 1,857 | 96 | 124 | 4,590 |
| | | | Min. | 133 | 505 | 13.1 | 378 | 336 | 636 | 70 | 112 | 566 |
| | | | Max. | 273 | 19,400 | 50.5 | 1,160 | 6,970 | 5,102 | 260 | 329 | 10,100 |
| | ♀ Juv. | 1 | | 285 | 1,150 | 54 | 114 | 237 | 4,604 | 1,967 | 6,321 | 323 |
| *B. buteo* | ♂ Mat. | 20 | Median | 364 | 1,040 | 46 | 330 | 244 | 950 | 1,960 | 1,920 | 2,000 |
| | | | SD | 360 | 1,720 | 204 | 176 | 727 | 1,000 | 2,654 | 4,286 | 1,980 |
| | | | Min. | 66.7 | 146 | 16.6 | 106 | 111 | 67.7 | 256 | 290 | 160 |
| | | | Max. | 1,222 | 6,100 | 930 | 840 | 2,292 | 3,162 | 9,300 | 16,600 | 8,200 |
| | ♂ Juv. | 2 | Median | 232 | 566 | 26.3 | 102 | 63,650 | 1,560 | 5,660 | 6,060 | 2,140 |
| | | | SD | 146 | 208 | 16.4 | 29.3 | 89,900 | 1,050 | 2,320 | 1,940 | 697 |
| | | | Min. | 129 | 414 | 14.8 | 80.8 | 164 | 820 | 4,000 | 4,700 | 1,680 |
| | | | Max. | 334 | 707 | 38 | 123 | 126,260 | 2,300 | 7,280 | 7,470 | 2,650 |
| | ♀ Mat. | 15 | Median | 410 | 1,200 | 48.5 | 306 | 320 | 1,750 | 1,960 | 4,800 | 1,790 |
| | | | SD | 250 | 8,890 | 2,475 | 390 | 2,200 | 1,190 | 4,343 | 5,560 | 2,000 |
| | | | Min. | 48 | 188 | 9.30 | 81 | 18.2 | 67.7 | 535 | 515 | 242 |
| | | | Max. | 808 | 35,300 | 9,600 | 1,326 | 8,890 | 4,750 | 12,500 | 19,600 | 7,475 |
| | ♀ Juv. | 1 | | 313 | 677 | 84.8 | 364 | 182 | 1,734 | 1,250 | 1,160 | 1,575 |
| *S. aluco* | ♂ Mat. | 11 | Median | 178 | 850 | 123 | 150 | 150 | 1,836 | 380 | 647 | 790 |
| | | | SD | 241 | 1,772 | 138 | 143 | 1,840 | 8,384 | 465 | 4,545 | 440 |
| | | | Min. | 43 | 71.7 | 20.6 | 81 | 98 | 552 | 178 | 244 | 180 |
| | | | Max. | 859 | 5,100 | 480 | 546 | 5,050 | 27,550 | 1,530 | 11,720 | 1,420 |
| | ♂ Juv. | 5 | Median | 263 | 1,170 | 126 | 168 | 170 | 2,756 | 580 | 970 | 412 |
| | | | SD | 152 | 253 | 55 | 107 | 135 | 11,820 | 618 | 677 | 408 |
| | | | Min. | 176 | 780 | 60 | 81 | 867 | 1,777 | 465 | 444 | 279 |
| | | | Max. | 566 | 1,394 | 170 | 323 | 380 | 26,000 | 1,590 | 1,940 | 1,160 |
| | ♀ Mat. | 11 | Median | 398 | 2,400 | 152 | 146 | 102 | 3,470 | 260 | 289 | 500 |
| | | | SD | 228 | 1,650 | 546 | 360 | 1,790 | 14,450 | 700 | 378 | 300 |
| | | | Min. | 12 | 25.6 | 29.6 | 80.8 | 18.2 | 246 | 33 | 47.5 | 152 |
| | | | Max. | 677 | 4,590 | 1,920 | 1,313 | 5,450 | 48,500 | 1,920 | 1,140 | 1,020 |
| | ♀ Juv. | 7 | Median | 55.5 | 150 | 116 | 174 | 130 | 1,428 | 606 | 560 | 778 |
| | | | SD | 178 | 269 | 101 | 566 | 134 | 1,500 | 656 | 370 | 566 |
| | | | Min. | 36.8 | 122 | 42.8 | 84 | 18.2 | 919 | 166 | 148 | 143 |
| | | | Max. | 490 | 828 | 303 | 1,530 | 398 | 4,850 | 1,920 | 1,160 | 1,560 |

*n* number of samples, *Mat.* mature, *Juv.* juvenile, *SD* standard deviation, *Min.* minimum value, *Max.* maximum value

metal concentrations between males and females are probably explained by similar dietary habits in the sexes and the low rate of excretion of metals in eggs.

Only significant correlations were observed between the concentration of Cd in kidney and feathers of each species (*A. gentilis* r = 0.782**, *B. buteo* r = 0.466**, *S. aluco* r = 0.345*) as well as between Cd in feathers and liver (r = 0.471**) of *B. buteo*. For Pb, only the correlation between liver and feathers was significant (r = 0.308*) in *B. buteo*, whereas for Hg none of the correlations were significant. By other hand, significant correlations were observed between hepatic and renal Cd (*B. buteo* r = 0.771**, *A. gentilis* r = 0.874** and *S. aluco* r = 0.951**), as well as Hg (*B. buteo* r = 0.890**, *A. gentilis* r = 0.993** and *S. aluco* r = 0.886**). Finally, significant liver-kidney relationship for Pb was found in *B. buteo* (r = 0.521**).

It is generally assumed that the concentrations of metals in feathers represent circulating levels in blood at the time of formation, as there is an active input of blood to the feathers (Goede and de Bruin 1984; Braune 1987). These metals may originate from recent exposure via the diet or from mobilization from tissues from where they were sequestered (Braune and Gaskin 1987; Lewis and Furness 1991). When the feather matures, the vascular connection atrophies and the feather remains as an indicator of the metal levels in the blood of the individual (Braune and Gaskin 1987).

Although some authors previously reported a certain degree of significant correlation between metal levels in feathers and other tissues (Gochfeld 1980; Ohlendorf et al. 1985), we only found significant correlations for the three species between the concentrations of Cd in kidneys and feathers -exceptionally other two significant correlations were obtained for *B. buteo*. This result was expected because the higher concentrations of Cd were found in the target organs, kidneys, than those found in liver. However, the correlation coefficients were generally too low and therefore we had to reject the possibility of using regression analysis to predict the concentrations of metals in the kidneys from the concentrations in feathers. The lack of significant correlations in most of cases and the low correlation coefficients may be because the feathers reflect the accumulation of heavy metals in the year prior to moulting. However, the viscera will reflect the concentrations of heavy metals obtained from the diet, and which will accumulate in these organs over time. In addition, the data on the adult birds was generally more variable than the data on the juvenile birds. Although there were no significant differences in the concentrations between the age groups, the adult birds comprised a highly heterogeneous group, with specimens ranging from aprox. 2 to 25 years old, probably due to the age of the birds. The difficulty in determining the exact age of the adult birds is therefore a disadvantage of the use of internal organs in biomonitoring studies with birds, as the extent to which the metal load is due to time of exposure or degree of contamination of the ecosystem cannot be established. Nevertheless, this problem does not exist when feathers are used as these reflect the metal concentrations eliminated each year, independently of the age of the individuals. The range of metal concentrations in feathers is also greater in birds than in other tissues, which suggests that they provide a reliable indication of the different metal concentrations in the environment. Finally, other advantages of the use of feathers are that they can be obtained non-invasively and they are easier to process than other tissues.

In conclusion, with regard to the use of *A. gentilis*, *B. buteo* and *S. aluco* as biomonitors of heavy metal contamination, the results show that feathers, compared with viscera, are the most appropriate tissue for analysis. However, further methodological studies must be carried out to establish which feathers are the most apt for biomonitoring studies and when samples should be collected.

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