Acta Veterinaria (Beograd), Vol. 58, No. 4, 315-324, 2008.

DOI: 10.2298/AVB0804315Z UDK 619:615.916

METAL CONCENTRATIONS IN THE LIVER AND KIDNEY OF RAPTOR SPECIES FROM THE CALABRIA REGION, ITALY

ZACCARONI ANNALISA*, ANDREANI GIULIA**, FERRANTE MC***, CARPENÈ E**, ISANI GLORIA** and LUCISANO A***

**Department of Veterinary Public Health and Animal Pathology, Bologna University, Italy*
***Department of Biochemistry, Bologna University, Italy*
****Department of Animal Pathology and Health, Napoli University, Italy*

(Received 16. November 2007)

We have focused our study on the concentrations of non essential trace metals Pb, Cd and Cr and essential heavy metals Zn and Cu, in the liver and kidney of raptor species from different areas of the Calabria region in Italy, with the aim of obtaining information concerning the quality and the contamination level of their habitat. No significant differences related to sampling areas and gender were found, while significant differences were found in metal concentrations among species. Mean Cd concentrations in the kidneys were generally 1.5-10 fold higher than hepatic ones, while Pb was mostly concentrated in the liver; a different pattern in tissue Cr concentration was observed between species. Referring to essential metals, Cu levels were higher in the liver, whereas higher Zn concentrations were constantly present in the kidneys. The ratio between kidney and liver metal concentrations (K:Ls) was also evaluated. The measured concentrations in toxic elements can be considered as indicative of chronic exposure to low amounts of pollutants. The essential metal concentrations were in the range of physiological values and did not show the presence of any deficiency or excess of metal. The present study can be considered as a starting point in the study of a possible correlation between levels of pollutants and the appearance of any adverse effects in raptor species in the Calabria area.

Key words: heavy metals, avian species, environmental monitoring, Italy

INTRODUCTION

Increased bioavailability of heavy metals to all organisms, connected with industrial, agricultural and urban activities, is responsible for accumulation of both essential, Zn and Cu, and non essential metals, like Cd, Cr and Pb. Being these metals unlikely to produce lethal effects, even if present at concentrations higher

than physiological ones, their transfer through different steps of the food-chain and their accumulation in top predators (principally raptors) is eased.

Quantification of metal in tissues of different raptors can allow the evaluation of their habitat's quality and contamination, as well as the assessment of the potential risk of exposure for studied species and humans.

Lot of work has been done to detect the effect of some metals, e.g. Pb, Hg, on avian species, while little is known concerning the majority of other elements. So, the risk linked to Pb shot pellets ingestion for waterfowls and raptors is well documented, as well as that of a possible Hg intoxication for sea birds (Wayland *et al*., 2005). On the other side, little information is available for Cd, Cr, Zn and Cu toxic levels found in wild species, except for acute episodes due to spilling or industrial accidents (MacDonald *et al*., 1983; Edwards *et al*., 1992). In some cases the toxic thresholds have been defined: Burgat (1990) defines values of 3-10 ppm as the toxic threshold for Cd, and Feierabend and Myers (1984) consider that Pb liver concentration below 6 ppm dry weight is the equivalent of exposure to "background" levels, while concentrations between 6 and 20 ppm dry weight indicate above background exposure, and concentrations higher than 20 ppm dry weight are indicative of acute exposure.

All this considering, it has been thought interesting to evaluate Pb, Cd, Cr, Cu and Zn in tissues of various raptors species (common buzzard, kestrel, barn owl, marsh harrier, tawny owl, little owl, sparrow hawk, honey buzzard), originating from different areas of Calabria region, with the aim of evaluating their metal burden and of obtaining information concerning contamination of their habitat.

MATERIALS AND METHODS

Animals

In present study 57 livers and 70 kidneys from animals (81) who died at the Centro Recupero of Fauna Selvatica located in Rende (CS, 39°19'58"80 N, 16°11'6"72 E), Italy (Figure 1), between 1994 and 1996 were used. The following species were available: common buzzard (*Buteo buteo*) (n = 29), kestrel (*Falcus tinniculus*) (n = 19), barn owl (*Tyto alba*) (n = 10), marsh harrier (*Circus aeruginosus*) (n = 6), tawny owl (*Strix aluco*) (n = 5), little owl (*Athene noctua*) (n = 6), sparrow hawk (*Accipiter nisus*) (n = 3), honey buzzard (*Pernis apivorus*) (n = 3) (Table 1).

The differences in sample numbers between the liver and kidney were due to the conservation status of carcasses, as not always organ collection was possible. Gender of animals was registered when possible, while the collection area was always reported. The collection method did not reflect, if partially, the real distribution of species considered; thus specimens cannot be considered a random sample of the populations.

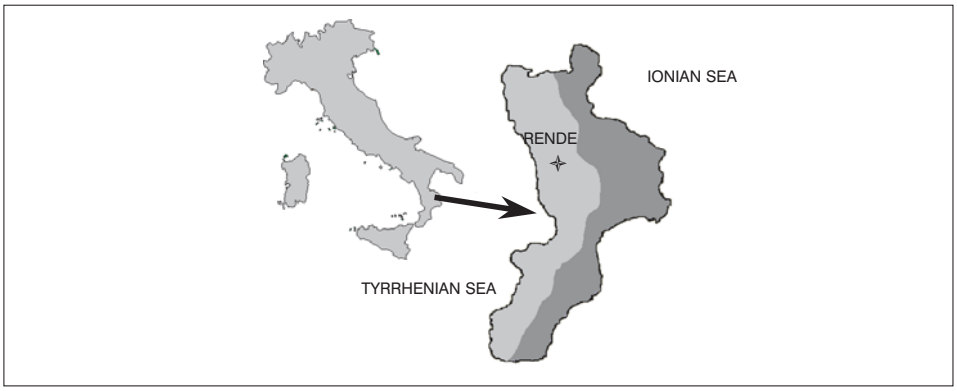

Figure 1. The map shows the location of the sampling region, with the two considered areas, Tyrrhenian and Ionian

Table 1. Ecological characteristics of studied species

Species	Weight (g)	Mammals	Birds	Amphibians	Reptiles	Insects	Fishes	Vegetables and honey
Barn owl n=10	M: 470 F: 570	X	x					
Common buzzard n=29	M: 690 F: 950	X	x		X	X		
Honey buzzard n=3	M: 810 F: 960	x	x	x	x	X		x
Kestrel n=15	M: 156 F: 193	X	x	x	x	X		
Little owl n=6	M: 105-200 F: 115-220	X	X	x	x	x		
Marsh harrier n=6	M: 500 F: 670	X	X	x	x	x	x	
Sparrowhawk n=3	M: 130-150 F: 250-320	x	X			x		
Tawny owl n=5	M: 410-550 F: 410-800	X	X			X		

X: main prey; x: minor prey.
Mean species weight was considered for dimensions.
For each species the number of specimens sampled is indicated.

Metal analysis

Analytical determinations were conducted on liver and kidney samples collected during necropsy and stored at –20ºC until analyses. Samples were freeze dried and 200 mg aliquots were subsequently mineralized following Angerer *et al*. (1988). The atomic absorption spectrophotometric method has been applied with a Perkin Elmer 2380 instrument with a graphite furnace for Cr (not for buzzard and marsh harrier) and Pb, and with a IL-11 Instrumentation Laboratories flame atomic spectrophotometer for Zn, Cu and Cd. All concentrations in tissues are expressed in ng/g or μg/g on a dry weight basis. All specimens were run in batches that included a blank, initial calibration standards and standard reference materials (CRM 278:lyophilized mussels). All values of reference materials were within certified limits given by the Community Bureau of Reference – BCR (Brussels). The detection limits were 1 ng/mL for Pb and Cr, 4 ng/mL for Cd and Zn, and 6 ng/mL for Cu.

Statistical analysis

To test for collection site effect, the Calabria region was divided in two principal areas (Tyrrhenian and Ionian) that are roughly separated by the mountains of the Appennino Calabrese (Figure 1). The effect of sampling area, gender and species on metal concentrations was analyzed by Variance Analysis using the one-way ANOVA test, using Mathematica® 3.0 Program (Wolfram Research Inc, Champaign, IL) and JMP 3.2.2 (SAS Institute, S. Francisco, CA).

Finally, the kidney to liver ratio (K:L) was calculated (Table 2), in order to evaluate the possible acute or chronic exposure of birds to metals. This ratio was calculated from the mean only for those animals in which both liver and kidney tissue samples were available (43 birds).

Table 2. Kidney:liver (K:L) ratio for each species considered

Species	Cd	Cr	Pb	Cu	Zn
Tawny owl	1.26	0.73	0.22	0.43	3.16
Barn owl	5.66	2.42	1.66	0.56	2.54
Little owl	14.68	1.51	1.06	0.94	1.28
Marsh harrier	1.80	n.a.	2.30	0.73	0.47
Honey buzzard	1.90	0.98	0.36	0.91	3.52
Kestrel	6.18	2.33	1.02	0.82	3.16
Common buzzard	2.44	n.a.	1.60	0.85	0.55
Sparrow hawk	2.74	1.47	0.45	0.64	2.38

n.a.: not available

RESULTS

Toxic and trace metal mean concentrations are summarized in Figures 2 and 3, respectively. No statistical differences were found when sampling area or gender were considered, both in liver and kidney. Only few statistical differences in metal concentrations were found among species, and only for the liver tissue.

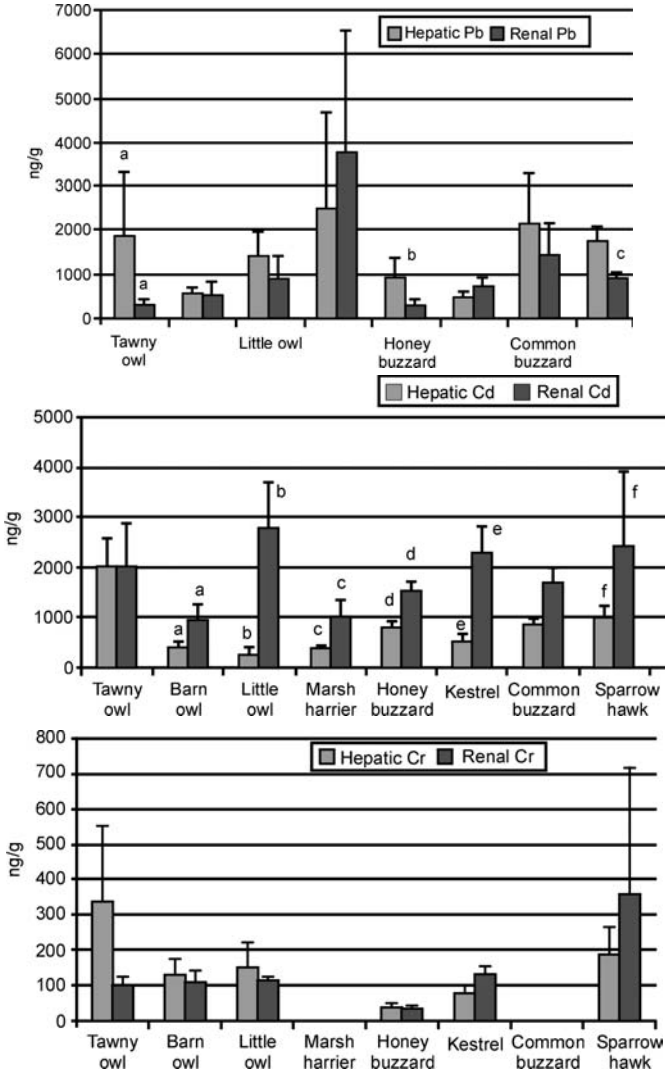

Figure. 2 Pb Cd and Cr concentrations in liver and kidney of raptors. Values are expressed as mean ± S.E.; values are expressed in nanograms per gram dry weight; values sharing the same superscript are significantly different, referred to tissue differences in same species

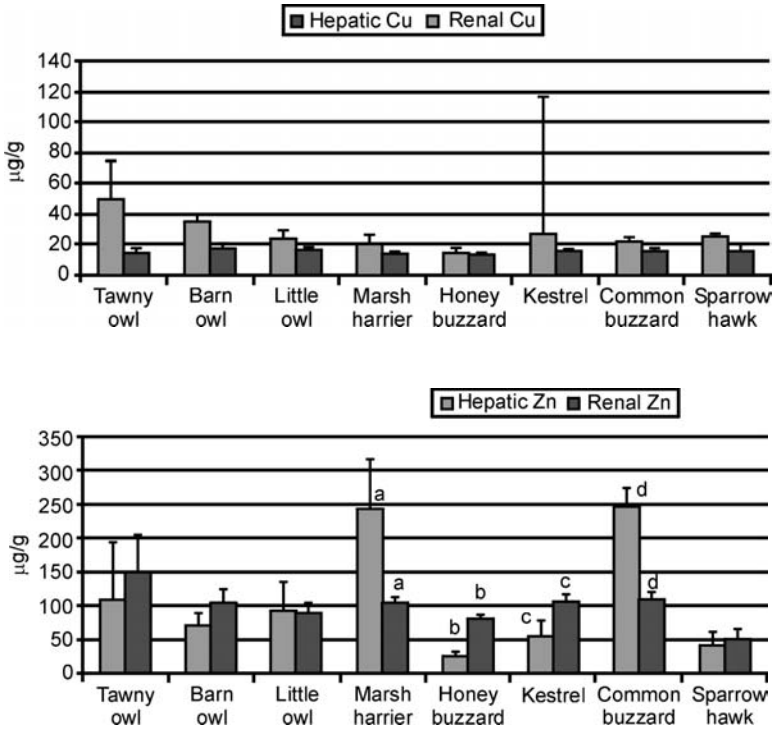

Figure 3. Zn and Cu concentrations in liver and kidney of raptors. Values are expressed as mean ± S.E.; values are expressed in micrograms per gram dry weight; values sharing the same superscript are significantly different, referred to tissue differences in same species

Toxic trace elements

Mean liver Pb concentrations in different species ranged from 311 ng/g d.w. to 2.52 μg/g d.w.; only few individuals in two species had Pb liver concentrations higher than 6 μg/g d.w. 2 out of 19 (10%) common buzzard and 1 out of 4 (25%) marsh harrier. Only one buzzard showed concentrations higher than 20 μg/g d.w. (21.48 μg/g d.w.). Mean Pb concentrations in the kidneys were generally lower than hepatic ones, marsh harrier and kestrel only showing mean values 1.5 and 2 folds higher than liver ones, respectively (Figure 2).

Hepatic mean concentrations for Cd were always below or close to 1 μg/g dry weight (d.w.) but in the tawny owl, where they reached values 2-10 times higher than in other species. The little owl shows the lowest concentrations, statistically different from those found in the sparrow hawk (5 folds higher, $P<0.05$) and common buzzards (4 folds higher, $P<0.05$). The common buzzard shows hepatic Cd mean values twice than those found in the barn owl. Renal Cd concentrations were 1.5-10 folds higher than hepatic ones, except in tawny owls, where values were similar (Figure 2). Statistical differences ($P<0.01$) were found

for Cd liver concentrations in the tawny owl vs. all other species, sparrow hawk vs. little owl, common buzzard vs. barn owl and little owl.

Cr always presents low concentrations, never exceeding 400 ng/g d.w. but in one sparrow hawk, where it reached renal concentrations of 714 ng/g d.w.. A different pattern in tissue concentrations could be observed between species, with some nocturnal raptors having higher liver levels, while diurnal raptors show higher renal levels (Figure 2).

Essential trace elements

Cu concentrations were generally higher in the liver of all species, ranging from 14.6 μg/g d.w. (marsh harrier) to 49.4 μg/g d.w. (tawny owl) in liver and from 12.7 μg/g (marsh harrier) to 17.1 μg/g (barn owl) in kidney samples (Figure 3).

Finally, Zn concentrations were always higher in the kidney; however, marsh harrier and common buzzard had higher Zn burden in the liver (about twice that of kidneys) while the little owl had comparable concentrations (Figure 3). Statistical differences ($P<0.01$) were found for Zn liver concentrations between the marsh harrier and common buzzard and all other species, but the tawny owl.

The species mean kidney:liver ratio (K:L) for all metals are summarized in Table 2. When Cd is considered, due to the significant accumulation of the metal in the kidneys, values higher than 1 were found for all the considered species with the exception of the tawny owl; the highest values (5.66-14.68) were obtained for those species living closer to urban areas (barn owl, kestrel and little owl). The K:Ls for Cr shows little variability, as well as those for Pb, which presents low values only for the tawny owl, honey buzzard and sparrow hawk. A great constancy could be observed for Cu, with values always below 1, and for Zn, with the only exception of the marsh harrier and common buzzard.

DISCUSSION

When looking at the present data, a certain attention should be paid in interpreting them, for there could be some bias which can alter the results. First, sampling is not random, for birds died of causes due to man, such as impact with man-made structures or shooting, may have a greater chance of being recovered than those that died of non-human related causes, such as diseases. Similarly, the scarcity of data concerning gender can partially explain the absence of statistical gender differences, even thought one can not exclude a real lack of different accumulation mechanisms, as detected by other authors (Wanntorp *et al.*, 1976; Peterson and Ellarson, 1976; Hoffman and Curnow, 1979; Hulse *et al*., 1980; Hutton, 1980; Hutton and Goodman, 1980; Fleming, 1981; Hutton, 1981; Parslow *et al*., 1982; Custer *et al*., 1986; Gochfeld and Burger, 1987; Zaccaroni *et al*., 2003, Carpenè *et al*. 2006).

When considering data as a whole, one can clearly see a certain species-specific difference in metal concentrations, which can be partially due to ecological differences. The biggest species prey on biggest targets, which can provide a higher burden of pollutants and this could be evident especially for renal levels of Cd. The presence of higher percent of mammals or birds in the diet can

also influence the metal burden for these predating species. The lack of differences when geographic area is considered can be indicative of a certain uniformity in exposure in the whole Calabria region.

Significant differences were referred to liver only. We partially explain these differences by considering that this organ is the one where toxicants are firstly accumulated and metabolized, while the kidney can be considered as an indicator for chronic exposure and this is mostly evident for Cd. In general liver and kidney can present high amounts of Cd due to their ability to synthesize metallothionein, which is first produced in the liver and then accumulated in the kidney for long term metal storage. The induction of this protein by Cd or Cu plays a fundamental role in the selective retention of those metals in some avian species, such as the woodcock (Carpenè *et al*., 2006).

Cd concentration in the analyzed animals are not indicative of an exposure to high levels of metal, even thought in some birds the K:L ratio seems to be indicative of a prolonged exposure to relatively high levels of Cd, as reported in ducks by White and Finley (1978), which found similar K:L ratios after feeding a diet up to 200 μg/g Cd for 3 months. A certain correlation with the level of anthropization of the species could be observed for Cd K:L the species closest to man, e.g. little owl, present highest K:L, thus indicating a possible higher chronic exposure to this metal. Some species show intermediate K:L values close to those found by White and Finley (1978) in the control animals. When Cr and Pb are considered, the K:L shows a great variability, which can hardly be explained by ecological differences. A possible explanation for such a variability could be the numerical differences between species, which can bias the correct estimation of the K:L value. Cu presents a very constant K:L, thus confirming the efficiency of the homeostatic mechanisms which control this essential metal. Finally, Zn shows a species-specific trend in K:L values, which could partially be explained with metabolic differences.

When comparing metal levels in each subject with defined toxic thresholds, some interesting data could be observed for Pb. Two buzzards and one marsh harrier could indeed be suspected of having been exposed to high environmental levels, while one buzzard could have died because of acute Pb intoxication. In all subjects ingestion of spent Pb shots could be suspected, thus confirming the important role of Pb shots as a source of intoxication.

CONCLUSIONS

Obtained data are comparable with those reported by other authors, which evaluated metal contamination in different raptor species and all indicative of a background exposure to the considered metals (Wiemeyer *et al*., 1987; Gochfeld and Burger, 1987; Carpenè *et al*., 1996; Garciá-Fernández *et al*., 1997; Battaglia *et al*., 2005). Obtained data for toxic elements can be considered as indicative of a chronic exposure to low levels of toxic metal contamination. Nevertheless, the data regarding 3 buzzards and 1 marsh harrier underline the importance of Pb shot pellet ingestion as a consequence of predation on hunter-killed or hunter-crippled waterfowl containing Pb pellets as a cause of intoxication for raptors. The

essential metal concentrations (Zn and Cu) detected in the present study are in the range of the physiological values and do not show the presence of any deficiency or excess. The present study can be considered as a starting point in the study of a possible correlation between levels of pollutants and the appearance of any adverse effects in raptor species in the Calabria area.

Adrress for correspondence:
Zaccaroni Annalisa,
Dept. Veterinary Public Health and Animal Pathology,
Bologna University,
Via Tolara di Sopra 50, 40064,
Ozzano Emilia, Bologna,
Italy
E-mail address: annalisa.zaccaroni@unibo.it

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KONCENTRACIJA METALA U JETRI I BUBREZIMA PTICA GRABLJIVICA IZ KALABRIJSKE REGIJE U ITALIJI

ZACCARONI ANNALISA, ANDREANI GIULIA, FERRANTE MC, CARPENÈ E,
ISANI GLORIA i LUCISANO A

SADRŽAJ

Naša ispitivanja su bila usmerena na određivanje koncentracije ne-esencijalnih metala: olova, kadmijuma i hroma kao i esencijalnih metala cinka i bakra u jetri i bubrezima ptica grabljivica iz različitih delova Kalabrije u Italiji. Osnovni cilj je bio određivanje stepena kontaminacije njihovih staništa. U ispitivanjima nisu utvrđene statistički značajne razlike u odnosu na pojedine delova Kalabrijske regije i pol jedinki, ali su postojale značajne razlike između pojedinih vrsta. Srednja koncentracija kadmijuma je u bubrezima bila 1,5 - 10 puta veća nego u jetri dok se olovo uglavnom koncentrisalo u jetri. U distribuciji hroma uočene su velike razlike između vrsta. Nivo bakra je bio veći u jetri a cinka u bubrezima. U studiji je takođe određivan odnos između koncentracija pojedinih metala u bubrezima i jetri (K : Ls). Izmerene koncentracije toksičnih metala su ukazivale na hronično unošenje malih doza, dok je koncentracija esencijalnih metala bila u fiziološkim granicama. Ova studija predstavlja početnu tačku za dalja ispitivanja nivoa polutanata u Kalabrijskoj regiji i njenim delovima.