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Pb, Cd, As, and Se Concentrations in Livers of Dead Wild Birds from the Ebro Delta, Spain

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Wild bird populations are susceptible to dangers derived from the environmental presence of toxic elements and substances, especially those that are non-degradable and that in many occasions tend to concentrate through the food chain. Monitoring of such substances in selected bird species from a delimited area could be useful not only to evaluate the health condition of the species involved, but also to assess the degree of contamination in the ecosystem where they live.

One of the most important wild bird sanctuaries of Europe, the Ebro Delta, is situated in NE Spain in the Tarragona province. The Ebro Delta, part of which is protected with the status of Natural Park, is an alluvial plain of about 32,000 ha, of which 7,736 ha corresponds to the Natural Park. Because of its special hydrological conditions and geographical situation, the Ebro Delta supports an important and diverse bird population, estimated at 180,000 in the winter period (January), and constituting 311 different species (Martínez Vilalta et al. 1989).

Most of the Ebro Delta's surface is dedicated to agriculture (24,500 ha), principally rice cultivation (19,000 ha), which also serves as an excellent wild bird habitat. Accordingly, agricultural pesticides could be a major problem for wildlife. But in the last few decades the Ebro River and the Tarragona coast have also undergone considerable industrial development. The area is now facing important environmental threats created by industrial activities, and as a result the ecosystem is becoming contaminated with heavy metals, petroleum hydrocarbons and PCBs. The literature has reported heavy metal concentrations in marine organisms of the zone (Schuhmacher et al. 1990), but data on metal and metalloid concentrations in birds were, until now, not available.

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The objective of this study was to monitor lead (Pb), cadmium (Cd), arsenic (As) and selenium (Se) concentrations in bird livers from diverse trophic levels in the Ebro Delta. Pb, Cd and As are well recognized as toxic elements for life, while Se, although causing well known deleterious effects for humans and domestic animals, has only recently been recognized as toxic for aquatic birds (Ohlendorf et al. 1986; Hoffman et al. 1988). The determination of values of contaminant loads in several avian species would be useful to control and prevent, when possible, pathological conditions derived from toxic levels of these contaminants for wild bird populations living in the Ebro Delta.

MATERIALS AND METHODS

All the birds were found dead or moribund in the area of the Ebro Delta between January and April 1989. Twenty-eight individuals of the following species were examined and analyzed: barn owl (Tyto alba) (n=1), black-headed gull (Larus ridibundus) (n=2), black-necked grebe (Podiceps nigricollis) (n=1), cattle egret (Bubulcus ibis) (n=11), flamingo (Phoenicopterus ruber) (n=2), gadwall (Anas strepera) (n=1), grey heron (Ardea cinerea) (n=2), herring gull (Larus cachinnans) (n=1), kingfisher (Alcedo atthis) (n=1), lapwing (Vanellus vanellus) (n=1), little egret (Egretta garzetta) (n=1), long-tailed duck (Clangula hyemalis) (n=1), mallard (Anas platyrhynchos) (n=1), mediterranean gull (Larus melanocephalus) (n=1) and pintail (Anas acuta) (n=1). The carcasses were weighed and then stored at -20°C at the Wildlife Rehabilitation Center of the Natural Park until necropsy. The sex, age and weight of the animals were recorded for each bird.

The causes of death were diverse, but many showed traumatic injuries due to impacts with man-made structures, resulting in bone fractures. One bird was found dead by gunshot and another was diagnosed with avian tuberculosis. The causes of death in the remaining birds was not fully established. It is important to remark that the animals selected for this study were limited to those collected during the four months mentioned and that were best conserved. Thus, they cannot be considered to be a random sample of the population, but represents those that died at the study area.

Following necropsy, the liver was removed from each bird and stored in polypropylene containers at -20°C. Care was taken to prevent external contamination of the specimens. Analyses were performed by atomic absorption spectrometry using a Perkin-Elmer Zeeman 3030 with an HGA-600 furnace and AS-60 autosampler. The stabilized temperature platform furnace (STPF) concept was utilized, including

atomization on a L'Vov platform inserted into a pyrolytically coated graphite tube, maximum power heat during atomization, peak area monitoring, and matrix modification to overcome interferences. Background correction was achieved by a magnetic field induced Zeeman effect. An electrode discharge lamp (EDL) was utilized for As and Se determinations, while a hollow cathode lamp (HCL) was used for the remaining analytes. Bovine liver and muscle and pig kidney (Promochem SRM 1577a, CRM 184, CRM 186), certified for Pb, Cd, As and Se, were used for recovery calculations and for validation of the techniques, with results ranging 90-110% the certified values in all cases.

Results are expressed in μg/kg liver wet weight. In order to compare the levels of these 4 elements with the diet of each bird, the typical food consumed by the 15 bird species was grouped in 4 types: plants (P), invertebrates (I), vertebrates (V) and rubbish and putrid flesh (RPF). P was considered as the lower or first trophic level, I the second level, V the third level, and RPF the higher or fourth trophic level. Information about the diets of the birds was obtained from several authors, and are displayed as percentages in Table 1. A Trophic Level Factor (TLF) was defined as the sum of the percentages of the four trophic levels, multiplying the value of the first level by a factor of 1, the second by 10, the third by 100, and the fourth by 1,000 (for example, the TLF of the barn owl is [0x1]+[0x10]+[100x100]+[0x1,000] = 10,000); the Relative Trophic Level (RTL) was defined here as the TLF ordered from higher to lower values in the 15 bird species examined in the present study.

Numerical analysis was performed using the SPSS/PC+ statistic package. In order to avoid the presence of "0" values for Pb, Cd and As, for all calculations the non-detectable (ND) concentrations were considered as one-half of the respective limit of quantification (i.e., 10, 2, and 25 μg/kg, respectively). Statistical evaluation consisted in a study of correlation (Pearson's correlation coefficient) between levels of elements and weight of the animals and TLF. The study of relationship between levels of the elements and the diet was carried out using the crosstabulation analysis and the χ^2 test of independence. The individual element concentration was treated at 2 levels, high and low: the higher groups were Pb>122, Cd>43, As>25 and Se>930; these values divided each set of data in two homogeneous groups with respect to the number of individuals.

RESULTS AND DISCUSSION

Table 2 shows the concentrations of the 4 elements in the liver of the 28 animals analyzed in this study. The

Table 1. Qualitative and quantitative estimation of the diet of the examined birds in the Ebro Delta.

Species	P	I	V	RPF	TLF	RTL
Barn owl	0	0	100	0	10,000	6
Black-headed gull	2	20	34	44	47,602	1
Black-necked grebe	0	93	7	0	1,630	9
Cattle egret	3	45	28	24	27,253	3
Flamingo	30	70	0	0	730	12
Gadwall	100	0	0	0	100	15
Grey heron	0	10	88	2	10,900	5
Herring gull	0	30	32	38	41,500	2
Kingfisher	0	20	80	0	8,200	7
Lapwing	8	92	0	0	928	11
Little egret	0	28	72	0	7,480	8
Long-tailed duck	0	100	0	0	1,000	10
Mallard	98	2	0	0	118	14
Mediterranean gull	5	30	49	16	21,205	4
Pintail	55	45	0	0	505	13
SUM	301	585	490	124		

P = plants; I = invertebrates; V = vertebrates; RPF = rubbish and putrid flesh. TLF = Trophic Level Factor; RTL = Relative Trophic Level.

concentrations ranged from ND to 12,460 μg/kg for Pb, ND to 497 for Cd, ND to 1,202 for As, and 394 to 2,050 for Se.

Except for the lapwing (bird #23), the remaining animals showed Pb concentrations that could be considered normal for birds, i.e., <2 μg/g wet weight (Friend, 1987). Primarily waterfowl and raptors are exposed to Pb poisoning through the ingestion of lead shots used by hunters (Friend, 1987; Pain, 1990), which is summed to the environmentally acquired Pb contamination. The lapwing of this study was clearly suffering Pb poisoning, but unfortunately its gizzard was not examined in search of pellet shots. In many places of the Ebro Delta hunting is permitted, and several bird species have been diagnosed as suffering from lead poisoning in this area (Guitart et al., unpublished data).

Concentration of Cd in bird livers shows some dispersion. Most of the liver samples contained levels comparable to previous studies in other countries: this is the case for the cattle egret, with a mean value in our study of 49 μg/kg, slightly lower than the mean values reported by Hulse et el. (1980) of 115 μg/kg, and by Husain and Kaphalia (1990) of 0.09 μg/g. The values reported in the last paper for other wild bird species are, however, lower than the concentrations found in the present study

Table 2. Lead, cadmium, arsenic and selenium (μg/kg wet weight) levels in liver of birds collected in the Ebro Delta during 1989.

Species	Code	Pb	Cd	As	Se
Barn owl	1	68	ND	ND	640
Black-headed gull	2	114	362	1,202	1,510
Black-headed gull	3	370	497	296	640
Black-necked grebe	4	194	43	251	1,100
Cattle egret	5	150	16	ND	394
Cattle egret	6	48	19	ND	483
Cattle egret	7	53	10	ND	700
Cattle egret	8	ND	39	ND	1,000
Cattle egret	9	130	86	ND	1,100
Cattle egret	10	ND	80	ND	920
Cattle egret	11	ND	97	ND	1,600
Cattle egret	12	ND	37	ND	620
Cattle egret	13	ND	26	ND	930
Cattle egret	14	104	66	ND	840
Cattle egret	15	198	66	ND	1,230
Flamingo	16	67	8	171	1,007
Flamingo	17	209	30	ND	1,025
Gadwall	18	1,063	122	ND	560
Grey heron	19	139	15	ND	707
Grey heron	20	353	24	ND	623
Herring gull	21	50	68	215	1,213
Kingfisher	22	160	50	ND	1,150
Lapwing	23	12,460	120	187	2,050
Little egret	24	122	47	533	1,120
Long-tailed duck	25	286	144	353	580
Mallard	26	ND	ND	ND	1,361
Mediterranean gull	27	326	308	1,045	2,000
Pintail	28	124	33	ND	900
MEAN		602	86	169	1,000
SD		2,333	116	299	421
MEDIAN		123	45	25	965

ND = Values below limit of detection: 20, 4, 50 and 15 μg/kg for Pb, Cd, As and Se, respectively. For statistical calculations when values are ND, see Methods.

for the black-headed gull and the Mediterranean gull. Nevertheless, the levels of Cd in birds of the Ebro Delta are similar or lower than those recently reported for edible marine fishes of the Ebro Delta coast (Schuhmacher et al., 1990).

As and Se have attracted a lot of attention in recent years due to the potential hazards to wildlife exposed to them as constituents of irrigation drainwater. The black-headed gull #2 and the Mediterranean gull showed levels above 1,000 μg/kg As, which can be considered remarkably

high levels, comparable to those obtained in young mallard ducklings receiving 100-300 μg/g As in the diet during 10 weeks (Camardese et al., 1990), doses that could adversely affect normal bird development.

Se is an essential trace element in animal nutrition, but at higher doses could produce toxic effects. In aquatic birds selenium toxicity is mainly manifested by teratogenesis and embryonic mortality (Ohlendorf et al., 1986; Hoffman et al., 1988). In the Kesterson Reservoir in California, livers of aquatic birds had mean Se concentrations ranging from 20 to 127 μg/g (dry weight) (Presser and Ohlendorf, 1987), which are clearly higher than the values obtained in the Ebro Delta. However, some values found in our study are slightly higher than some reported for birds from unpolluted areas (Presser and Ohlendorf, 1987).

The initial statistical evaluation showed that the levels of Cd and As were clearly correlated ($r = 0.68$, $p < 0.00001$), and also Se was correlated with Pb ($r = 0.47$) and As ($r = 0.49$), in both cases with a $p<0.02$. It must be remarked that all these correlations were positive, so the concentration of these elements seemed to increase together. However, when As is implicated, it may be remembered that 19 out of 28 of their values are in fact below the limit of quantification. Interestingly, a positive correlation ($r = 0.48$, $p<0.001$) was found between Cd and TLF, that was not observed with the other heavy metal we analyzed, neither when all the Pb values were considered nor when value #23 (12,460 μg/kg) was exluded from calculations based on the results of the outliers analysis.

When compared to the diet, it was found that levels of Pb showed a dependence in a negative sense with the consumption of rubbish and putrid flesh ($p<0.03$). This is probably due to the lead shots, that in some areas of the Delta are present at high densities (Guitart et al., unpublished data). They could represent the main source of lead in the zone, being more available to animals of the lower trophic levels.

The levels of As showed a positive dependence with the consumption of invertebrates ($p<0.02$), and although not significant at a 95% level also with the consumption of vertebrates ($p=0.068$). In many wetlands, contaminated fresh water is the source of As, and in those cases, As tends to accumulate at higher levels in plants. Although it cannot be demostrated with the present data, our findings suggest that the source of As in the Delta is not the water of the Ebro River, but probably has a terrestrial or marine origin.

As a conclusion of this study, levels of the four elements measured were, with few exceptions, within the range found in birds and other animals from zones other than the Ebro Delta. However, some individuals showed potentially toxic concentrations of metals and metalloids. Surveillance and monitoring of these and other elements should be, in our opinion, continued in this area for birds in all trophic levels.

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