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Accumulation of heavy metals and As in wetland birds in the area around Doñana National Park affected by the Aznalcollar toxic spill

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Abstract

The impact of the spill from the mine in Aznalcollar (Seville, Spain) on waterfowl in the Doñana National Park is assessed. The concentrations of Cu, Pb, Cd, Zn and As in the liver and eggs of 16 species of waterfowl found dead in the Park between April and November 1998 were determined. The highest levels were found for Zn, followed by Cu, Pb, Cd and As. The main parameters related to the accumulation of these elements in the waterfowl studied were species and trophic level. The other variables studied — distance from the spill, days of exposure, sex, size, and age — are important, although this depends on the element studied. Zn and Cu from the spill have entered the food chain of the aquatic birds studied, but Cd, Pb and As have not. There is currently no evidence to suggest that the trace element concentrations measured have reached toxic levels. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Acid mine waste; Heavy metals; Arsenic; Doñana National Park; Waterfowl

1. Introduction

The Doñana National Park (DNP) is a protected area that serves as a wildlife sanctuary for thousands of sedentary and migratory birds, which nest and in some cases live there temporarily. These birds offer an abundance of suitable biotopes for many species, including predatory birds and mammals. The DNP is a major centre of abundance in Europe and a nesting area for waterfowl species. Like other nature reserves in the European Union, the site needs to be protected as far as possible from contamination. Although this remarkable ecosystem was given official protection in 1969, having survived relatively

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[1]Dedicated to the memory of Luis Manuel Hernández.

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unspoiled for centuries thanks to the absence of permanent settlements, it has always suffered the impact of human activities. One of the most serious has been the mining of pyrite ores in Aznalcollar, rich in heavy metals and metalloids. Various studies carried out in the area between 1982 and 1992 showed that levels of heavy metals in water and soil had increased over this period in the northern area of the Park (Cabrera et al., 1984; González et al., 1985, 1990; Rico et al., 1989; Fernández et al., 1992; Arambarri et al., 1996). This increase has been linked to periods of mining activity and the various mineral extraction systems. Thus, in 1984, mining activities altered the pH of the water of the Guadiamar River, keeping it below pH 4 almost as far downstream as the point where it enters the DNP (Vado del Quema) (González et al., 1990). This caused a redistribution of heavy metals in the area and consequently an increase in the levels of heavy metals in the Guadiamar River from the mine to the entrance to the DNP. This is a continuous danger to the ecosystems in the surrounding area. Nevertheless, earlier studies showed that metal contamination in birds which feed in the wetlands was low (González et al., 1984; Rico, 1985; Hernández et al., 1986, 1987, 1988; Fernández, 1992), except in the case of Pb. The high levels of Pb found in birds in the DNP has always been linked to hunting in the area (Ramo et al., 1992; Mateo et al., 1998). The sudden avalanche of water and sludge produced by the collapse of the tailings dam storing mining waste from the Aznalcollar mine on the 25 April 1998 caused the rivers Agrio and Guadiamar to burst their banks, flooding approximately 4500 ha of adjacent land (Fig. 1). The amount of sludge deposited in the Guadiamar basin has been estimated at 5 million cubic meters. The contaminating sludge waste contains 0.5% arsenic, 0.8% lead and zinc, 0.2% copper, and 0.007% cadmium on a dry weight basis, and part of these metals is likely to pass into the food chains of organisms living in the DNP (Pain et al., 1998).

Wild bird populations are susceptible to dangers from toxic elements, especially those that are non-degradable. Environmental contamination from heavy metals and As is a threat to the survival and reproduction of bird populations (Ohlendorf et al., 1986; Scheuhammer, 1987; Stanley et al., 1994). Birds likely to be particularly exposed to high concentrations of metals include waterfowl and waders, which have a tendency to ingest soil and sediment with their food.

The data presented here are the preliminary results of extensive follow-up on the effects of metal contamination from the toxic spill at the Aznalcollar mine on living organisms in the DNP and the surrounding area. In particular, this paper reports heavy metal contaminants and arsenic in eggs and liver from waterfowl feeding in DNP marshes. Sixteen aquatic bird species from six families were selected (Anatidae, Rallidae, Laridae, Recurvirostridae, Threskiornithidae, and Phoenicopteridae) and dead specimens were collected in the area affected by the spill. These species are useful bioindicators of contamination of the aquatic environment, where they forage on the aquatic environment, feeding mainly on plants and invertebrate fish living in sediments and sludge. The levels of heavy metals found in the liver and eggs of waterfowl species after the mining accident were compared with those obtained from similar species living in the area between 1979 and 1982 as well as with those corresponding to similar species from other wetlands.

2. Material and methods

2.1. Sampling

One hundred and forty-seven livers and 35 infertile eggs from 16 waterfowl bird species (Table 1) were collected between April (just before the accident) and November 1998 in the Doñana Natural Park and surrounding area. Liver was chosen as soft tissue in which to analyse trace elements for two main reasons: firstly it is one of the soft tissues where the elements studied are mainly concentrated (Wenzel and Adelung, 1996), and secondly it is the only soft tissue for which data are available on heavy metal concentration in aquatic birds in DNP (Rico, 1985; Fernández, 1992). The specimens selected for this study were limited to those found dead during the months

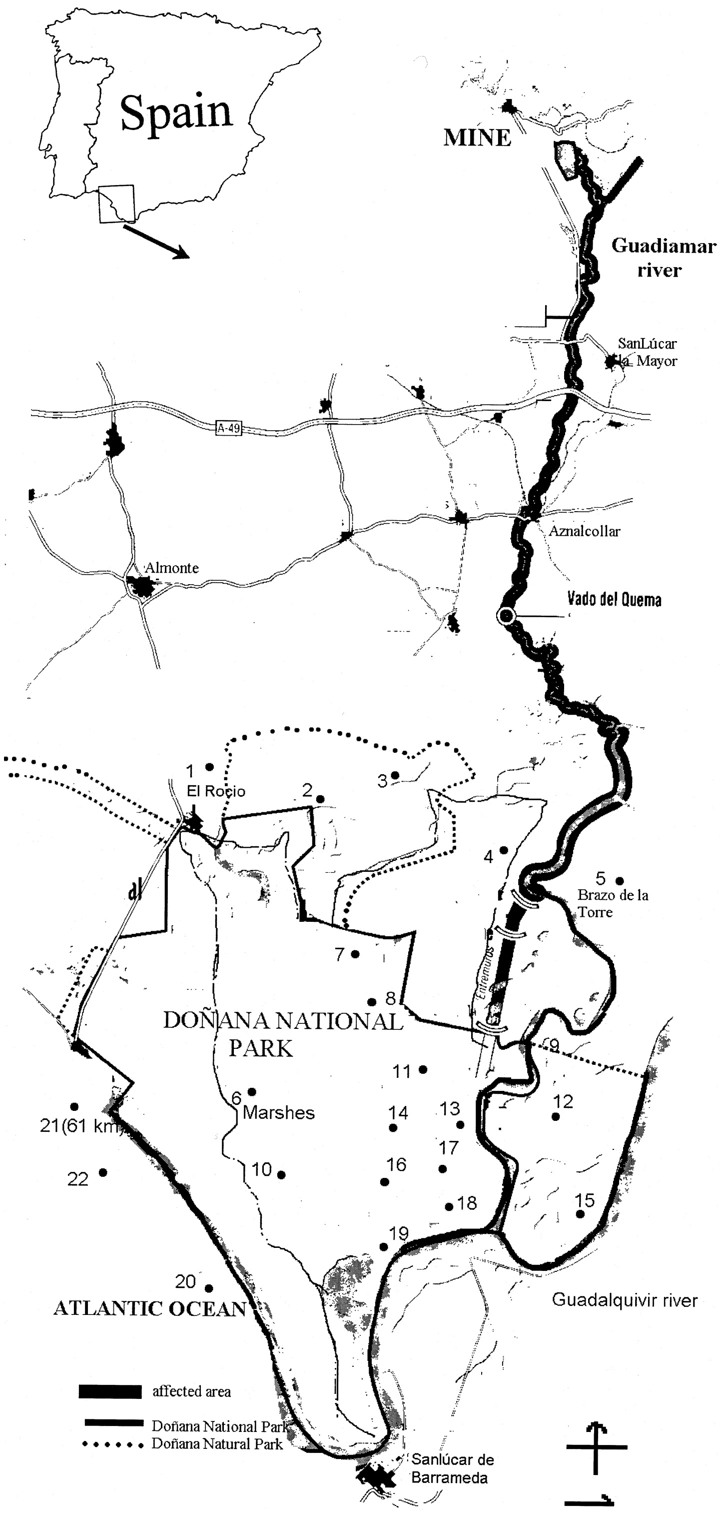

Fig. 1. Geographical location of the sampling points were the birds and eggs were collected.

Table 1
Characteristics of waterfowl species from DNP studied

Family[a]	Species		No of samples and type	Sampling point (see Fig. 1)	Days of exposure (range)	Type of food (trophic level)
	Common name	Scientific name				
Anatidae	Mallard	*Anas platyrhynchos*	10 livers	4[b]	72–89	Omnivorous
	Pochard	*Aythya ferina*	11 livers	4[b]	62–87	Omnivorous
	Teal	*Anas crecca*	5 livers	10, 15	126–192	Omnivorous
	Shoveler	*Anas clypeata*	12 livers	15	145–192	Omnivorous
	Gadwall	*Anas strepera*	17 livers	4[b], 16	65–89	Omnivorous
Rallidae	Coot	*Fulica atra*	24 livers	4[b], 15, 16	69–192	Herbivorous
	Purple gallinule	*Porphyrio porphyrio*	10 livers	11, 16	66–89	Herbivorous
	Moorhen	*Gallinula Chloropus*	3 livers	4[b], 5[b]	22–76	Omnivorous
Laridae	Whiskered tern	*Chlidonias hybrida*	24 livers, 6 eggs	11, 12,13 18	44 (eggs)	Fish predator
	Slender-billed gull	*Larus genei*	18 eggs	14	38	Fish predator
	Audouin's gull	*Larus audouinii*	2 livers	20	153	Fish predator
	Black-headed gull	*Larus ridibundus*	7 livers	4[b]	68–94	Crab and fish predator
Recurvi-rostridae	Black-winged still	*Himantopus himantopus*	16 livers	4[b]	64–108	Invertebrate predator
	Avocet	*Recurvirostra avosseta*	1 liver, 13 eggs	15, 17	44 (eggs)	Invertebrate predator
Threskior-nithidae	Spoonbill	*Platalea leucorodia*	1 liver	4[b]	89	Crab and fish predator
Phoeni-copteridae	Greater flamingo	*Phoenicopterus ruber*	3 livers	7[b], 3, 21	107–137	Invertebrate predator

[a]According to the Green Book.
[b]Sampling point affected by the spillage.

mentioned. Thus, they cannot be considered to be a random sample of the population. Autopsy of most of death aquatic birds gathered in Doñana National Park in 1998 was done. The agents causing their death were identified as bacteria affecting breathing and fungus (*Candida* sp.) (Galke, 1999). A natural mortality occurs in the region especially during the dry season. Annual mortality is highly variable depending on the drought cycles, summer temperature and infection agents (Avril et al., 1992; Galke, 1999). Table 1 indicates the main characteristics of the species studied, sampling points, days of exposure to the spill, number of specimens analysed, and feeding habits. Fig. 1 indicates the geographical location of the sampling points where the birds were collected. Sampling points 4, 5 and 7 were in the area affected by the accident and the others mainly lay within a radius of 3–10 km. The birds were stored at -20°C after collection until the analytical determinations were performed.

The 8 months of sampling (from April to November) were divided into two different periods. The first was from April to the 15 June 1998, when the acid waters, which flooded the land close to the Park, prevented the birds from feeding normally in the contaminated area. By the 15 June the acidity of the water had decreased considerably, and the birds were able to feed in the area affected by the spill, where the invertebrate fauna was beginning to recover.

2.2. Analytical procedure

Livers and eggs (albumen + yolk) were analysed for trace elements. An aliquot of each sample was oven-dried at 60°C to constant weight to calculate the water content. For element determination, 0.8 g of fresh tissue was placed in hermetic Teflon digester containers. Digestion was performed in an acid medium with HNO_3 and H_2O_2, with the sample in the furnace at 90°C for 4 h. Analyses of Zn, Cu, Pb and Cd were performed using a flame atomic absorption spectrometer (AAS) (Spectra A-100, Varian). Arsenic in eggs was measured using a Perkin Elmer (PE) longitudinal AC Zeeman (AAnalyst 600) AAS equipped with a transversely heated graphite atomiser and a built-in fully computer-controlled AS-800 autosampler (Perkin Elmer Hispania, S.A., Madrid, Spain). PE pyrolitic graphite-coated tubes with an inserted L'vov platform were used. For the determination of arsenic in liver a PE Model 3300 atomic absorption spectrometer equipped with a PE flow injection analysis system for atomic spectroscopy (FIAS-400), an autosampler (PE AS-90) and a electrothermally heated quartz cell were used. Concentrations are expressed in μg/g on a dry weight basis. To convert to wet weight basis the concentration should be divided by 3.46 or 3 (arithmetic mean of water content) for liver and eggs, respectively. The instrumental detection limits (LOD) were Cd (0.01 μg/ml); Cu (0.03 μg/ml); Pb (0.2 μg/ml); Zn (0.01 μg/ml) and As (0.08 μg/ml). All specimens were analysed in batches, with method blanks, known standards, and reference material, DORM-2 [dogfish liver (*Squalus acanthias*)], and TORT-2 [lobster hepatopancreas), from NRCC. Accepted recoveries of reference material ranged from 88 to 110%. Relative standard deviation (R.S.D.) in replicates and reference material was always below 10%.

2.3. Statistical analysis

The distribution of data was highly skewed in most cases, the variables were transformed to natural logarithms to obtain a normal distribution. The data set was analysed by analysis of variance (ANOVA) to compare the two time periods. Significant differences between arithmetic means were determined by the Student's t-test when non-significant differences between variances were observed, and by the Cochran t-test when the variances were different (Snedecor and Cochran, 1975). A Statistical Statgraphic package (version.5.0, STSC Inc., Rockville, Maryland, USA), was used for the calculations. The non-detectable (ND) concentrations were considered as one-half of the respective limit of detection (LOD).

Generalized Linear Model, or GLM (Nelder and Wedderburn, 1972; Dobson, 1983; McCullagh and Nelder, 1983), was used to derive a mathematical description of the individual variations of the concentrations of the various elements in liver. Generalised Linear Models are a class of models of which linear regression is a particular case. Three components have to be defined for a GLM: a linear predictor, an error function, and a link function.

A linear predictor (LP) is defined as:

$$LP = a + b * x_1 + c * x_2 + \dots,$$

where a is a constant to be estimated; b, $c, \dots$ are parameters to be estimated from empirical data; and x_1, $x_2 \dots$ are the explanatory variables. The error function depends on the nature of the data set. The concentration of the various elements in liver was ln-transformed and a normal error distribution was assumed for the models.

An identity link was used as the link function. In this case the model does not differ from a multiple linear regression with the dependent variable (liver metal and As concentrations) ln-transformed.

The explanatory variables considered were introduced into the model as factors (species, sex, trophic position) or continuous variables (days of exposure to the spillage, distance from the source of the spill, and size).

The explanatory variables were fitted to the observed data by a modification of the traditional forward step-wise procedure in response to criticisms that have been levelled at this kind of fit (Donázar et al., 1993; Bustamante, 1997; Forero et al., 1999).

The expression of the model becomes:

$$[\text{metal}] = e^{a + b * x + c * x + \dots}$$

When a large part of data related to Cd and As was not available or the metals were not detectable, some of the species that appear in Table 1 were not included in the models. The species concerned are, for Pb: whiskered tern, spoonbill, purple gallinule, and black-headed gull; and for As: whiskered tern, avocet, and spoonbill.

3. Results

Table 2 shows the arithmetic mean concentration and ranges, in μg/g dry wt., of the five trace elements in 147 livers and 35 eggs from 16 waterfowl species living in the DNP and the surrounding area over three different periods: before the spill (between 1975 and 1992), from the time of the accident to the 15 June 1998, and from the 15 June 1998 to November 1998. Those species for which only a single specimen was available (avocet and spoonbill) were not included in the discussion of results. These values were considered an indication of the levels of metals in these two species. The highest liver concentrations were found for Zn, followed by Cu, Pb, Cd and As, considering the period between April and November 1998. Concentrations ranging from 2.916 to 1084 μg/g were found for Zn, from 2.054 to 1298 μg/g for Cu, from nd to 24.28 μg/g for Pb, from nd to 10.78 μg/g for Cd, and from nd to 5.394 μg/g for As.

The livers of common coot, common pochard and gadwall presented the highest levels of the four heavy metals and arsenic. Very high values of Cd and As were found in the gulls (black-headed and audouin's), Zn and Cu in moorhens and flamingos, and Pb and Cd in the terns.

The arithmetic mean of the five elements found in unfertilised eggs collected in the area in June 1998 were very similar for the three species sampled (avocet, slender-billed gull, and whiskered tern) (Table 2). As these samples were collected in areas remote from the spill (sampling sites 12, 14, and 17), and before the 15 June, the effects of the spill were hardly detected.

In order to explain the concentration of Zn, Cd, Cu, Pb, and As in liver, two GLM groups were fitted. In the first group (Model 1) seven variables (species, trophic level, days of exposure, distance from the spill, sex, age, and size) were included, and in the second group (Model 2) variables were reduced to six. Various significant models were found in both groups, except for Pb in the second group of models, as none of the variables considered were found to be significant for this metal. A single model — the one with the lowest residual variance — was selected in each group (Table 3). In the case of Zn, the only factor that contributed significantly in Model 1 was species. When this factor was eliminated (Model

Table 2
Arithmetic mean and/or range concentrations in μg/g dry wt.[a] in liver and eggs of waterfowls from DNP in three periods of time[b]

Family and species	Sample	Cu			Cd		
		1975–1992[c]	April–15 June (1998)	15 June–Nov. (1998)	1975–1992[c]	April–15 June (1998)	15 June –Nov. (1998)
Anatidae				90.07	(1) 0.120		0.165
Mallard	Egg	(1) 6.840					
Mallard	Liver			(10) 5.068–181.5			(10) nd–2.178
Pochard	Egg	(17) 3.660–10.38			(17) nd–0.780		
Pochard	Liver			478.5			0.537
				(11) 52.11–1298			(11) nd–1.270
Teal	Liver			35.20			0.466
				(5) 14.86–49.66			(5) nd–1.762
Shoveler	Liver			29.90			0.21
		(3) 24.53–40.66		(12) 10.03–59.03	(3) 0.727–5.190		(12) nd–0.838
Gadwall	Egg	(7) 3.090–6.330			(7) 0.120–0.270		
Gadwall	Liver			114.5	(1) 1.350		1.269
				(17) 8.352–288.7			(17) nd–6.610
Rallidae							
Coot	Egg	(5) 3.150–4.170			(5) nd–0.180		
Coot	Liver	(1) 47.30		149.9	(1) 0.420		0.294
				(24) 12.63–498.3			(24) nd–0.858
Purple gallinule	Liver			24.23			(10) nd
				(10) 5.627–50.22			
Moorhen	Liver		(1) 21.20	45.42		(1) nd	0.838
Laridae				(2) 27.01–63.84			(2) 0.077–1.598
Whiskered tern			1.974			0.186	
	Egg	(4) nd–10.44	(4) 1.530–2.460		(4) nd – 0.780	(4) nd–0.660	
Whiskered tern	Liver		13.16			1.228	
			(26) 7.750–31.69			(26) 0.484–3.564	
Slender-billed	Egg		0.330			(18) nd	
gull		(2) 2.460–8.730	(18) nd–1.350		(2) 0.180–0.360		
Audouin‘s gull	Liver			9.720			6.070
				(2) 8.509–10.93			(2) 1.354–10.78
Black-headed	Liver			35.91			1.737
gull				(6) 2.054–64.63			(6) 0.592–3.283
Recurvirostridae				16.09			1.913
Black-winged stilt	Egg	(2) 18.220–9.780			(2) 0.150–0.510		
Black-winged stilt	Liver			(16) 3.730–25.80			(16) nd–3.580
Avocet	Egg		1.320			(13) nd	
		(2) 4.620–7.740	(13) nd – 2.370		(2) nd–0.300		
Avocet	Liver	(1) 27.33		(1) 33.27	(1) 2.280		(1) 2.126
Threskiornithidae							
Spoonbill	Egg	(24) 2.370–22.39			(24) 0.150–0.770		
Spoonbill	Liver	(6) 27.37–228.8		(1) 43.69	(6) 0.270–0.880		(1) nd

Table 2 (*Continued*)

Family and species	Sample	Zn				Pb		As	
		1975–1992[c]	April–15 June (1998)	15 June–Nov. (1998)	1975–1992[c]	April–15 June (1998)	15 June–Nov. (1998)	April–15 June (1998)	15 June –Nov. (1998)
Phoenicopteridae									
Greater flamingo	Egg	(10) 3.426–3.975			(10) 0.120–0.190				
Greater flamingo	Liver	(3) 35.96–94.04		83.64 (3) 74.99–91.71	(3) 0.790–1.690		(3) nd		
Anatidae									
Mallard	Egg			(1) 0.630					
Mallard	Liver			163.0 (10) 65.70–364.9			0.131 (10) nd–0.482		0.226 (10) nd–0.495
Pochard	Egg	(17) 43.71–91.65			(17) nd–8.400				
Pochard	Liver			342.2 (11) 152.9–515.5			6.480 (11) nd–24.28		0.309 (10) nd–0.552
Teal	Liver			83.91 (5) 54.13–98.43			0.812 (5) nd–2.344		0.377 (5) 0.162–0.811
Shoveler	Liver	(2) 137.9–298.8		65.32 (12) 46.63–93.44	(2) 3.391–8.788		1.649 (12) nd–6.708		0.406 (12) 0.092–0.762
Gadwall	Egg	(7) 66.88–100.4			(7) 2.130–4.320				
Gadwall	Liver			296.2 (17) 95.51–615.7	(1) 6.610		3.788 (17) nd–15.01		0.165 (16) nd–0.547
Rallidae									
Coot	Egg	(5) 42.09–87.72			(5) nd–2.700				
Coot	Liver	(1) 189.4		288.6 (24) 108.9–1084	(1) 5.400		2.656 (24) nd–8.703		0.630 (18) 0.174–5.394
Purple gallinule	Liver			98.80 (10) 2.916–173.3			(10) nd		0.125 (10) 0.156–0.290
Moorhen	Liver		(1) 444.5	355.2 (2) 250.4–459.9		(1) nd	0.044 (2) nd–0.077	(1) nd	(2) nd
Laridae									
Whiskered tern	Egg	(4) 39.66–65.10	8.618 (4) 23.58–28.86		(4) nd – 2.410	0.102 (4) nd–0.360			
Whiskered tern	Liver		88.59 (26) 55.08–132.0			4.014 (24) nd–10.97		(8) 0.727	
Slender-billed gull	Egg	(2) 59.28–63.90	9.270 (18) 17.40–38.37		(2) 2.160–3.780	2.64 (13) 0.290–5.000		0.160 (13) nd–1.900	
Audouin's gull	Liver			55.66 (2) 55.11–56.20			0.450 (2) nd–0.873		3.012 (2) 2.893–3.130
Black-headed gull	Liver			184.9 (6) 88.29–269.4			(6) nd		0.176 (3) nd–0.308

Table 2 (*Continued*)

Family and species	Sample	Zn				Pb		As	
		1975–1992[c]	April–15 June (1998)	15 June–Nov. (1998)	1975–1992[c]	April–15 June (1998)	15 June–Nov. (1998)	April–15 June (1998)	15 June –Nov. (1998)
Recurvirostridae Black-winged stilt	Egg	(2) 47.01–66.39		142.5	(2) 2.010–4.110		0.298		0.119
Black-winged stilt	Liver			(16) 65.25–302.5			(16) nd–1.120		(11) nd–0.359
	Egg		10.70			0.480		0.060	
Avocet		(2) 39.90–74.85	(13) 22.02–66.90		(2) nd–6.060	(10) 0.280–1.200		(10) nd–1.190	
Avocet	Liver	(1) 211.1		(1) 300.7	(1) 11.07		(1) nd		
Threskiornithidae									
Spoonbill	Egg	(24) 17.64–53.10			(24) 0.380–10.32				
Spoonbill	Liver	(6) 132.4–364.9		(1) 152.2	(6) 2.210–4.050		(1) nd		
Phoenicopteridae									
Greater flamingo	Egg	(10) 42.45–56.21			(10) 1.830–2.240				
Greater	Liver			203.4			0.885		0.685
flamingo		(3) 138.7–189.1		(3) 121.2–281.0	(3) 5.330–11.00		(3) nd–2.614		(3) nd–1.460

[a]The concentration in dry weight have been calculated by multiplying the values of the eggs and the livers by 3 and 3.46, respectively.
[b]Number of individuals analysed are giving between parenthesis. nd, not detected; na, not analysed.
[c]González et al. (1984), Hernández et al. (1986, 1987, 1988), Fernández (1992), Rico (1985).

Table 3
Variables (factors and continuos) contributing significantly in the GLM models. In Model 1 all studied variables were included and in Model 2 the variable 'species' has been eliminated

Element	Model 1			Model 2		
	Accounted deviance	Factors	Continuous variable	Accounted deviance	Factors	Continuous variable
Zn	68.36	Species	–	12.36	Trophic	Distance
Pb	8.63	Species	–	–	–	–
Cd	51.11	Species age	 –	44.89	Trophic	Size
Cu	54.82	Species	–	19.66	Trophic	Size
As	60.47	Species	distance	46.49	Trophic	Days size

2), one factor — trophic position — and one continuous variable — distance — became significant. This model predicts a decrease in Zn liver concentration with increasing distance from the site of the spill (Table 5). For Pb, the variance explained was lowest in the first group of models, in which species was the only factor with a significant effect. Species and age were the most significant factors for Cd. Chick Cd concentrations were lower than those of the adults for all the species considered (Table 4). In the second group of models, the variables that entered sig-

Table 4
GLM models for element trace concentrations in aquatic bird livers, including all studied variables, with normal error and identity link

Model 1	Cu		Cd		Zn		Pb		As	
	Estimated parameter	S.E.	Estimated parameter	S.E.	Estimated parameter	S.E.	Estimated parameter	S.E.	Estimated parameter	S.E.
Constant	1.139	0.296	−0.883	0.560	3.196	0.196	−4.605	2.055	−3.435	0.270
Pochard	2.875	0.390	−1.402	0.756	1.238	0.257	1.552	2.146	0.756	0.354
Coot	2.210	0.342	−2.759	0.673	1.093	0.226	1.698	2.097	1.497	0.306
G. flamingo	2.093	0.568	−3.289	1.094	0.913	0.375	1.492	2.373	0.253	0.524
Gadwall	1.853	0.359	−2.275	0.661	1.126	0.237	2.212	2.114	−0.161	0.292
Mallard	1.811	0.398	−3.658	0.742	0.480	0.263	0.702	2.155	0.320	0.354
Spoonbill	1.311	0.890	−3.520	1.682	0.501	0.588	–	–	–	–
Moorhen	1.067	0.568	−2.431	1.063	1.425	0.375	0.231	2.373	−0.594	0.497
Avocet	1.049	0.890	1.261	1.682	1.193	0.588	0.000	2.055	–	–
Shoveler	1.025	0.383	−2.778	1.716	−0.116	0.253	2.037	2.139	1.123	0.312
Teal	0.993	0.478	−2.347	0.896	−0.007	0.316	1.556	2.251	0.529	0.396
Black-headed gull	0.726	0.453	0.326	0.845	0.717	0.299	–	–	0.371	0.488
Purple gallinula	0.644	0.398	−3.520	0.731	−1.550	0.263	–	–	0.000	0.270
Black-winged	0.264	0.363	0.105	0.681	0.501	0.240	0.688	2.118	−0.622	0.347
Audouin's gull	0.077	0.663	0.085	1.662	−0.226	0.438	1.688	2.517	2.666	0.577
Whiskered tern	0.000	0.296	0.000	0.560	0.00	0.196	–	–	–	–
Distance									0.041	0.0142
Age (pul)			−0.894	0.365						
Res. deviance	19.06		126.02		10.08		0			1286.7
d.f.	114		112		114		93			91

nificantly were trophic position and size, as factor and continuous variable, respectively. According to this group of models, fish predators would present the highest concentrations of Cd in liver and herbivorous birds would have the lowest level, increasing for all trophic levels as the size of the bird decreases (Table 5). For Cu, the only variable with a significant effect in the first group of models was species. In the second group, trophic level affected the model significantly as a factor and size as a continuous variable. That is to say the metal concentration increased with size at all trophic levels (Tables 4 and 5). For As, in Model 1 species was significant as a factor, and distance as a continuous variable; for all species, the concentration of As in liver increased with the distance from the spill (Table 4). In the second group of models three variables were found to be significant: one factor, trophic position, and two continuous variables, days of exposure and size. For all trophic levels the concentration of this metal increased with the number of days of exposure to the spill and the size of the specimens.

In order to simplify the comparison of the results among the three periods studied, species have been grouped by families (Anatidae, Laridae, Recurvirostridae, Rallidae, Phoenicopteridae and Threskiornithidae) (Table 1).

Percentages of specimens of each species exceeding the maximum values in the range corresponding to the two earlier periods, either in the same species or, if data for a particular species are not available, in the same family, are shown in Fig. 2. The maximum value was exceeded by 50% of the specimens in seven out of the 16 species studied in the case of Cu, in five species in the case of Zn, in three species in the case of Cd, and in one species in the case of Pb.

Considering all the species together, the averages of Zn and Cu increased significantly (Zn: $t = 17.22$, $P < 0.05$; Cu: $t = 14.52$, $P < 0.05$) with time. On the other hand, the arithmetic mean concentrations of Pb and Cd decreased significantly (Pb: $t = -21.89$; $P < 0.05$; Cd: $t = -17.34$; $P < 0.05$). No previously published results for As were available for comparison. However, an increase in the As levels was observed in some of the species analysed during the study. This increase could be related to the spill. The As levels found in the liver of the black-winged stilt collected in the contaminated area (sampling point 4) increased from < 0.02 to 0.36 μg/g (dry wt.) from June to August 1998.

4. Discussion

Results obtained indicate that Cu and Zn from the mine spill are contaminating the waterfowls of DNP. This is particularly serious in the case of

Table 5
GLM models for trace elements in aquatic bird livers, not including the species as variables, using normal and identity link

Model 2	Cu		Cd		Zn		Pb		As	
	Estimated parameter	S.E.	Estimated parameter	S.E.	Estimated parameter	S.E.	Estimated parameter	S.E.	Estimated parameter	S.E.
Constant	2.393	0.2218	−3.443	0.3342	3.603	0.1525	–	–	−3.499	0.2573
Omnivorous	0.156	0.2265	0.734	0.3412	0.482	0.1876	–	–	−0.593	0.1806
Herbivorous	0.000	0.2218	0.000	0.3342	0.000	0.1525	–	–	0.000	0.2573
Fish and crayfish predator	−0.088	0.4195	2.659	0.6669	0.309	0.3674	–	–	−0.342	0.4962
Fish predator	−1.040	0.2955	2.661	0.4450	0.307	0.2446	–	–	−1.356	0.2606
Invertebrate predator	−1.331	0.3954	2.854	0.5955	−1.198	0.3191	–	–	2.02	0.6032
Days									0.0081	0.0019
Distance					−0.274	0.0113				
Size	0.0005	0.0002	−0.0013	0.0003					0.0005	0.0001
Res. deviance	19.06		103.76		10.08		–	–	1286.7	
d.f.	124		123		123		–	–	97	

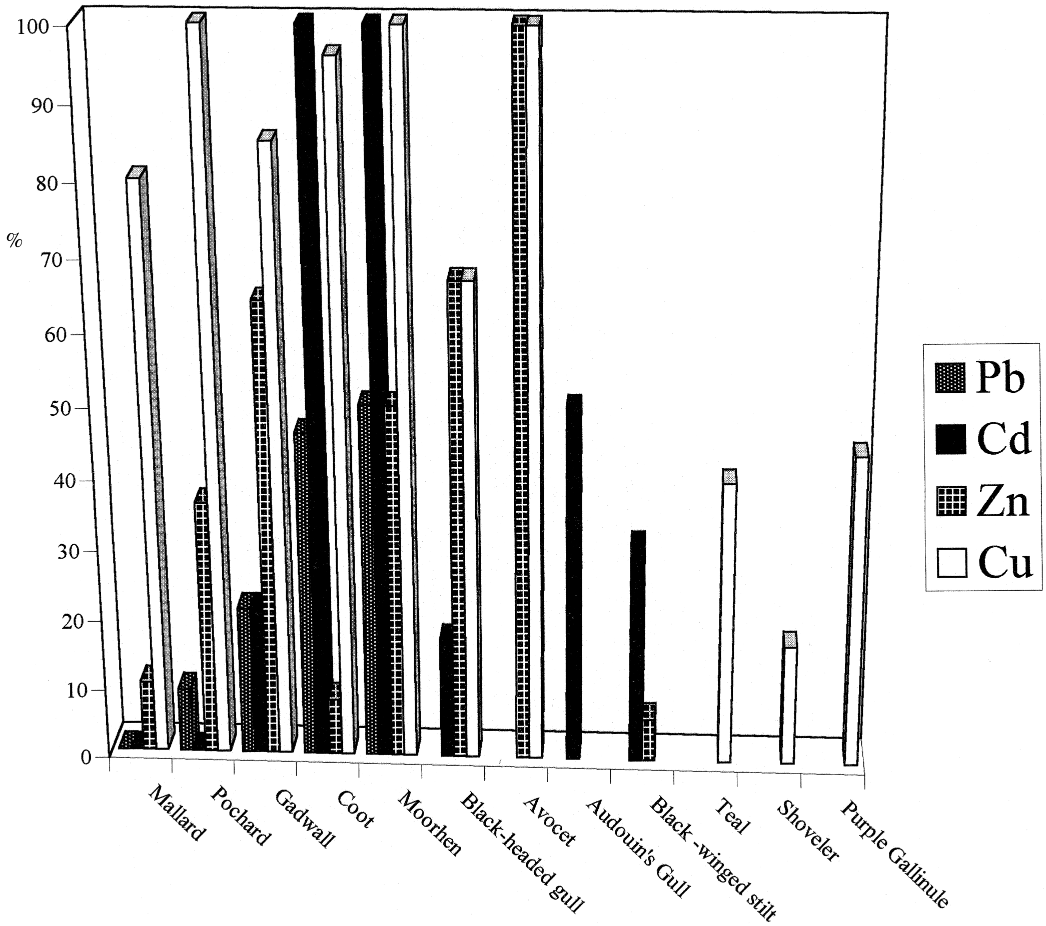

Fig. 2. Percentage of specimens of each species that exceed the maximum value in the range of element concentrations found before the period from the 15 June to November 1998.

Zn. The GLMs fitted for this metal revealed an increase in Zn contamination of individual specimens as the point of capture approached the spill area. This correlation was not so apparent for the other elements studied. GLMs (Tables 4 and 5) showed that the levels of Cd in liver increased with age, which could be related to a prolonged low-level chronic exposure (García-Fernández et al., 1995, 1996). The main route by which Cd entered in the wetlands is the Guadalquivir River, which passes through large industrial and urban areas such as Seville before reaching the wetlands (Rico et al., 1989; Fernández et al., 1992; Ramos et al., 1994).

The levels of Pb were lower than levels detected before the spill. As high concentrations of the metal were found in the sludge from the mine, this result suggests that Pb has not yet reached the food chain. In contrast to Cd, very high Pb levels were detected in some of the gulls studied. Earlier studies (Ramo et al., 1992; Mateo et al., 1998) showed that birds in the DNP suffered from chronic to sublethal poisoning by this metal due to intensive hunting in the area. The observed reduction in the levels of Pb mainly reflects the effect of its prohibition in the Park from 1984.

The trends for As were not so clear. The GLMs showed that, as expected assuming the mining spill to be the source of contamination, there was a general trend towards an increase in levels with the number of days elapsed since the accident. However, surprisingly, the same models showed that the As contamination tended to increase away from the area affected by the spill. This apparently surprising result can be explained by the fact that the water pH increased with the distance from the spill, which made As more soluble and consequently increased its availability for intake. Similar trends were observed in the concentration of As in the blood of other species (Benito et al., 1999, this volume). Following the implications of the results quoted above, we conclude that: (i) there might be an additional source of As contamination in the area; and (ii) As from the spill has not yet entered the food chain.

The GLMs showed that when a specimen of a given species was considered separately from its trophic position, species was the factor which best explained the variability observed in the concentration of metals and As in liver. This may be bound up their ability to absorb and excrete metals and/or their ecological characteristics (Leonzio et al., 1986; Ohlendorf and Fleming, 1988; Guitart et al., 1994a,b). In some species of mammals, the level of contamination by metals has been explained by the inability to metabolise them. However, we cannot rule out the possibility that the importance of species was connected with ecological factors such as longevity, ability to move elsewhere, differences in microhabitat and feeding. These factors were not included in the study, but have been recognised to affect the level of contamination (Marsili et al., 1995).

When species was eliminated from the model, the second most important variable was trophic level. Trophic level, in connection with feeding habits, was found to be the determining variable in the accumulation of a metal in the liver when individuals of various species were considered (Leonzio et al., 1986; Ohlendorf and Fleming, 1988).

The levels of Cd, Cu, and Zn reported here in the eggs of waterfowl (Table 2) were similar to those previously reported (Hothem and Welsh, 1994; Burger and Gochfeld, 1995; Mora, 1996; Gochfeld, 1997; Morera et al., 1997; Sanpera et al., 1997). These values were also lower than those found in the DNP from 1975 to 1992 (González et al., 1984; Hernández et al., 1987, 1988). The concentration of Pb in eggs reported here was generally higher than those reported for other areas (Burger and Gochfeld, 1995; Gochfeld, 1997), but similar to or even lower than those published for similar species living in the DNP from 1975 to 1992 (Hernández et al., 1986, 1987, 1988). Therefore, there is no evidence to suggest an increase in the trace element concentrations measured in the DNP. All values reported here were lower than those regarded as toxic in the literature (Ramo et al., 1992; Mateo et al., 1998). Pb concentrations in liver (Table 2) were usually higher than those previously published for similar species (Guitart et al., 1994a,b; García-Fernández et al., 1995; Llacuna et al., 1995; Eaden and Schoonbee, 1996), in both polluted and unpolluted wetlands. In addition, some values were higher than 7 μg/g (dry wt.), which is the upper threshold of expected background levels in waterfowl (Pain et al., 1996) and the lowest limit at which waterfowl Pb poisoning might prove fatal (Locke and Thomas, 1996). However, they were far from the 38 μg/g (dry wt.) which is considered to cause serious health effects in aquatic bird species (Locke and Thomas, 1996).

The Zn and Cu concentrations in liver reported in this paper were much higher than those found in waterfowl in other wetlands (Michot et al., 1994; Llacuna et al., 1995; Eaden and Schoonbee, 1996). These values were only comparable to those detected in wetlands in the Gulf of Mexico (Mora and Anderson, 1995). However, they were much lower than levels considered toxic (Eisler, 1993). Guillemot chicks can tolerate up to 2000 μg/g zinc (Johnson et al., 1962; Ewan, 1978) and up to 300 μg/g copper (National Academy of Sciences, 1980) without showing any toxic effects. Doneley (1992), however, observed moderate to severe nephrosis in caged and aviary birds containing zinc levels of 320 μg/g and 534 μg/g dry wt., respectively. Both values were below levels detected in some species in this study.

Cd and As were within the ranges usually reported in other studies (Guitart et al., 1994a;

Michot et al., 1994; García-Fernández et al., 1995; Llacuna et al., 1995; Mora and Anderson, 1995), which included both wetlands highly affected by contamination (industrial, agricultural or urban) and those theoretically free of contamination. Background levels are assumed to be less than 3 μg/g dry wt. for Cd and 5 μg/g dry wt. for As (Di Giulio and Scanlon, 1984; Pedersen and Myklebust, 1993). These levels were far below those considered toxic. Toxic effects of Cd only occur in humans and other mammals when liver and kidney concentrations reach approximately 30 μg/g dry wt., which is well above the concentration recorded for birds in this study (Scheuhammer, 1987). As concentration levels were also lower than those causing adverse effects in bird development (Camardese et al., 1990).

None of the levels attained by the elements studied were close to levels that can give rise to toxic effects in the birds studied. However, these heavy metals levels were relatively high, which shows that the target birds were exposed to metals in their environment. In general, it is difficult to link metal levels directly with reproductive success, and no effect so far has been detected. Species and trophic level were found to be the key factors determining heavy metals and As detected in the 16 species of waterfowl studied. The other variable studied — distance from the spill, days of exposure, sex, age, and size — have a much lower influence.

Evaluation of possible sublethal effects due to these elements, including pollutants and other stress factors, may warrant further research. Results presented here argue in favour of continuous monitoring of metals and metalloids in birds from the DNP, as well as an increase in the number of species and individual specimens examined, due to the enormous variability of the results obtained so far.

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