Available online at www.sciencedirect.com
SCIENCE DIRECT®
Environmental Research 100 (2006) 349–361
Environmental Research
www.elsevier.com/locate/envres

After the Aznalcóllar mine spill: Arsenic, zinc, selenium, lead and copper levels in the livers and bones of five waterfowl species

M.A. Taggart[a,*], J. Figuerola[d], A.J. Green[d], R. Mateo[c], C. Deacon[a], D. Osborn[b], A.A. Meharg[a]

[a]*Department of Plant and Soil Science, University of Aberdeen, Cruickshank Building, Aberdeen AB24 3UU, UK*
[b]*The Centre for Ecology and Hydrology, Monks Wood, Abbots Ripton, Huntingdon, Cambridgeshire PE28 2LS, UK*
[c]*Instituto de Investigación en Recursos Cinegéticos,CSIC-UCLM, Ronda de Toledo s/n, 13005 Ciudad Real, Spain*
[d]*Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avenida de Maria Luisa s/n, 41013 Sevilla, Spain*

Received 16 February 2005; received in revised form 19 July 2005; accepted 28 July 2005
Available online 13 September 2005

Abstract

In April 1998, a holding lagoon containing pyrite ore processing waste, failed and released 5–6 million m^3 of highly polluting sludge and acidic water. Over 2650 ha of the internationally important Doñana Natural Park became contaminated, along with <100 ha of the more pristine Doñana National Park. In order to assess the affect of the spill on waterfowl from Doñana, bone and liver samples from 124 individuals have been analysed for As, Pb, Cu, Zn and Se. Five species have been studied, from the Rallidae (rails), Anatini (dabbling ducks) and Aythyini (pochards) families. Geometric mean bone concentrations 2–3 months after the spill were in the order of Zn > Cu > Pb > Se > As, while liver concentrations were in the order of Zn > Cu > Se > Pb > As. Dry weight bone concentrations ranged from n.d-1.76 mg kg^{-1} As, 109.4–247.6 mg kg^{-1} Zn, 0.06–1.27 mg kg^{-1} Se, n.d-134.11 mg kg^{-1} Pb, and 2.18–8.92 mg kg^{-1} Cu. Wet weight liver concentrations ranged from n.d-0.34 mg kg^{-1} As, 29.8–220.1 mg kg^{-1} Zn, 0.15–0.85 mg kg^{-1} Se, n.d-3.80 mg kg^{-1} Pb, and 7.30–742.96 mg kg^{-1} Cu. The most important factor related to the accumulation of these metals was commonly species; however, location and sex also had important effects on liver As levels, location and age affected Cu levels, while Zn and Pb were affected by age, sex and location. Birds from Natural Park areas were found to have significantly higher levels of bone Zn, Pb and Cu, and liver As and Cu than birds from National Park areas. Female birds had higher liver As, Zn and Pb than males; whilst adults appeared to have lower bone As and Zn but higher liver Pb than chicks/juveniles. Although metal concentrations were elevated in certain individuals, in the majority of birds studied, they did not reach levels widely considered to be toxic. However, it would appear that As and Cu liver levels (which may be indicative of short-medium term pollutant exposure) were elevated in waterbirds which died in the spill contaminated Natural Park, 2–3 months after the disaster.

Keywords: Arsenic; Heavy metals; Aznalcóllar; Waterfowl; Liver; Bone

"**Capsule**": 2–3 months after the Aznalcóllar mine spill, Cu and As appeared to have entered the waterfowl food chains of the Doñana Natural Park.

*Corresponding author. Fax: +44 (0) 1224 272703.
E-mail addresses: mark.taggart@abdn.ac.uk, soi445@abdn.ac.uk (M.A. Taggart).

0013-9351/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2005.07.009

1. Introduction

On the 25th of April 1998, a massive holding lagoon containing pyrite ore processing waste failed, and released an estimated 5–6 million m^3 (Vidal et al., 1999; Galán et al., 2002) of acidic, metal rich sludge and water into the Rio Guadiamar, SW Spain. The Rio Guadiamar flows through Doñana, one of the most important conservation areas for birds in Europe (partly protected as a World Heritage Site, Biosphere Reserve and Ramsar Site), in which 70% of all European bird species can be found. Despite rapid emergency response to the disaster, some 2754 ha of the Doñana Natural/National Park sustained some degree of contamination. The Natural Park areas suffered the greatest impact, with 2656 ha affected (Grimalt et al., 1999). Of this, a 900 ha area known as the 'Entremuros' was most heavily impacted (Meharg et al., 1999; Taggart et al., 2004, 2005). An extremely important Natural Park zone (Fig. 1), this area acts as a seasonal wetland and therefore a rich breeding ground for many bird species, including some (i.e, marbled teal (*Marmaronetta angustirostris*)) that are globally threatened (Pain et al., 1998).

Waste from the spill entered the Entremuros, a 22 km long and 1 km wide 'bunded' area (within which water levels can be partially controlled) from the north. Although sludge impacted only the most northerly reaches of this feature, contaminated water reached as far south as Dam 2 (see Fig. 1). The sludge component of the waste contained 0.4–0.6% As, 0.8–1.3% Pb, 0.5–0.9% Zn, 0.1–0.2% Cu and 0.001–0.002% Se (Galán et al., 2002; Pain et al., 1998; Alastuey et al., 1999). The acidic, metal rich waters of the spill were stored temporarily, in the Entremuros (protecting more pristine National Park areas further south). The pH of the waters in this important wetland environment fell from 8.5 to 4.5, and in open water, Zn levels were recorded up to 270 mg L^{-1}, Pb up to 2.5 mg L^{-1}, Cu $<$0.01 mg L^{-1}, As up to 0.011 mg L^{-1} and Se $<$0.005 mg L^{-1} (Pain et al., 1998; Garralón et al., 1999). Fish and invertebrate populations were decimated, and valuable habitat, especially for waterfowl, was destroyed. Emergency clean-up operations began immediately, sludge was removed, and contaminated water treated and discharged. However, the scale of the disaster meant that residual contamination inevitably remained (Galán et al., 2002; Taggart et al., 2004, 2005), and the longer term impacts on the ecosystems of Doñana are yet to be determined.

Doñana is considered to be a very important haven for resident and migratory bird species. Of those birds, waterfowl, which feed in the wetlands contaminated by the Aznalcóllar spill, are perhaps at greatest risk from the longer term transfer of metal contaminants through the Doñana wetland food chains. Herbivorous waterfowl may be especially susceptible, as they have a tendency to consume significant amounts of soil/sediment associated with their food (Beyer et al., 1994, 1999; Beyer, 2000). Beyer et al. (1999) reported that soil ingestion by herbivorous grazing ducks was 3%, and higher than in granivorous filtering species, which ingested $<$1%. Waterfowl may serve as important bioindicator species, useful in terms of monitoring the long term impacts of the spill. They are also important, in that certain species (e.g, greylag geese (*Anser anser*)) are a key food source, not only for predators such as the highly endangered Spanish Imperial Eagle (*Aquila adalberti*), but also for humans who hunt and consume ducks, geese and rallids in the area.

Prior to the spill, a number of studies determined metal and organochlorine levels in bird eggs in Doñana, (González et al., 1984; Hernández et al., 1986, 1987,

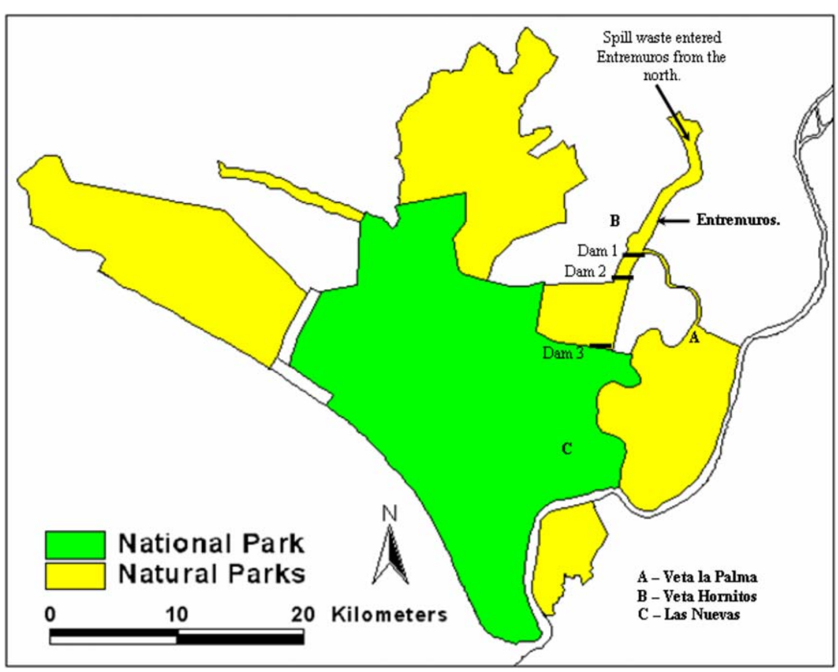

Fig. 1. Map of Donana Natural/National Park areas showing the location of the Entremuros and the areas from which the majority of samples were collected.

1988a, b), although these studies focussed on predatory species (Falconiformes and Ciconiformes) prone to egg shell thinning due to OC contamination in their food. These studies concluded that of those metals analysed, (typically Pb, Cu, Hg, Zn and Cd), metal levels in eggs were generally below that which would cause concern. More recently, elevated Pb levels in tissues of certain waterfowl and raptors from Doñana, have been linked to the historic use of lead shot for hunting in the area (Mateo and Guitart, 2003; Mateo et al., 1998, 2000, 2001a). Certain waterfowl species may consume (accidentally or otherwise) significant quantities of shot when foraging for grit. This is used to grind up food in their gizzards, and these species are in turn, important prey items for certain raptors (Mateo et al., 2001a, 2003).

Since the Aznalcóllar accident, Benito et al. (1999) and Hernández et al. (1999) have shown that metal levels in the blood, liver and eggs of birds in Doñana did not generally reach toxic levels soon after the spill, but that Zn, Cu, Pb and Cd levels did appear to be elevated in relation to uncontaminated areas. Meharg et al. (2002) and Pastor et al. (2001, 2004), both working on white storks (*Ciconia ciconia*), have also shown, using blood Pb isotope and 'Comet assay' techniques, respectively, that Pb from the spill has been taken up by white storks, and that long term genetic damage can be found. Recently, Gómez et al. (2004) also reported that, 2 years after the spill, an apparent increase in Cd, Pb, Cu, Zn and As could be detected in the tissues of waterfowl from Doñana National Park.

The lack of comprehensive 'pre-spill' monitoring data in Doñana makes it difficult to be conclusive when discussing the impact of the spill on metal levels in birds. Along with the Benito et al. (1999), Hernández et al. (1999) and Gómez et al. (2004) studies, the data included herein should provide a valuable starting point from which to assess the future, longer term impacts of the spill, on birds in the area. This study is the first to present data on 'bone' metal concentrations, Se levels in bone and liver matrices, and to consider, in detail, the effect of age and sex on metal levels in waterfowl soon after the spill. None of the individuals included in this study have been analysed in previously published papers. In a broader context, this paper provides valuable information on the levels of five metals in five waterfowl species, in both liver and bone matrices, and discusses these levels in relation to species, family and feeding habits, sex, age and geographical location.

2. Methods

2.1. Sampling

One hundred and twenty-four birds from 5 species were collected between the 2nd of June and the 26th of July 1998, 2–3 months after the mine spill occurred. In addition to these samples, data is also presented herein on 11 purple gallinules, (*Porphyrio porphyrio*). These specimens are dealt with separately within the paper since eight (6 of which were chicks) were found before the spill in July 1997 and February 1998, and 2 were collected in February 2000. Data is however given, and discussed, because this species is considered to be of 'European conservation concern' due to its rarity (Tucker and Heath, 1994) and is resident in significant numbers in the heavily impacted Entremuros area. The eight specimens found before the spill were collected in relatively pristine National Park areas, and could therefore be considered to represent birds relatively unaffected by either the mine spill, historic mining pollution inputs, and other anthropogenic inputs in the area, (i.e, light industrial and agricultural). The 2 specimens found in 2000 were collected in Veta La Palma, an area of Natural Park adjacent to those affected by the spill, and by historic pollution inputs from other anthropogenic sources.

As all of the birds studied here were found dead, they should not be considered an unbiased population sample. However, necropsies routinely carried out on birds found dead in Doñana in 1998 generally found metal poisoning was not the direct cause of death, but that death was commonly due to bacteria affecting breathing, and certain fungal infections (*Candida* sp.) (Galke, 1999).

Although location was routinely recorded when dead birds were found, these were often very general, and therefore, for the purposes of the analysis presented herein, location is simply split into Natural and National Park. Only a small part, 98 ha (Grimalt et al., 1999), of the relatively pristine National Park was affected by the spill, and this area has been historically well protected from anthropogenic pollution. The Natural Park sustained a greater impact, with 2656 ha affected. The Natural Park areas, as well as being badly affected by the spill, have historically acted as a buffer zone around the National Park. This has meant that they have also received a certain degree of pollutant impact from other anthropogenic activities. Fig. 1 shows the boundaries of the Natural and National Park areas. Twenty five birds have been analysed from the Natural Park, and 99 from the National Park. Of the 25 Natural Park birds, 1 was from the contaminated Entremuros, 3 were found in the adjacent Veta La Palma, and 21 were from Veta Hornitos, <2 km from the contaminated area. Of the 99 National Park birds, 74 were from Las Nuevas, 5–10 km from the contaminated area.

After collection, birds were necropsied, and sex, age, date found, location found, species, and the number of ingested lead shot in the gizzard were recorded. Samples of liver, femur and humerus were taken, and liver samples were stored frozen until analysis, while bone samples (with marrow removed) were allowed to air dry, and stored in desiccators until analysis. The number of lead shot in the gizzard were recorded as this has been shown to affect Pb and other metal concentrations in bird tissues (Mateo and Guitart, 2003). However, none of the birds analysed herein contained any visible lead shot in their gizzards. Use of Pb shot within Doñana was prohibited in 1984 (Hernández et al., 1999). Thirty-eight females, 86 males, 32 chicks, 56 juveniles and 36 adults have been studied. 'Chicks' were born in the breeding season in which they were found but were not fully grown (although they were all relatively large chicks, over half the length of the adults). 'Juveniles' were born in the breeding season in which they were found but were fully grown and fully winged. 'Adults' were >1-year old.

2.2. Laboratory procedures

Approximately, 0.5 g of liver or bone (humerus) was removed from the bulk sample using a stainless steel scalpel or stainless steel bone cutters, and weighed to an accuracy of 0.001 g, into an acid cleaned digest tube. To each digest tube, 2.5 mL of analytical grade 70% nitric acid was added, the tubes covered, and left overnight to digest. In total, 2.5 mL of analytical grade 30% hydrogen peroxide was then added and the digest tubes heated in a heating block, in 10° steps up to a final temperature of 160 °C. This temperature was then maintained for 4 h, until the sample was fully digested. The digest solution was then decanted into 15 mL PP centrifuge tubes, and made up to 10 mL with de-ionised water. These solutions were stored under refrigerated conditions until analysis.

Cu and Zn concentrations in the digests were determined using a Perkin-Elmer AAS 100; arsenic and Se were determined using a Perkin-Elmer Hydride Generation AAS 300 system; Pb was determined using a Perkin-Elmer Graphite Furnace AAS 3300 system. Arsenic was determined after pre-reduction of an aliquot of the sample with a solution of 10% potassium iodide, 10% hydrochloric acid, and 5% ascorbic acid; while Se was analysed after acidification of an aliquot of sample with 10% hydrochloric acid. All liver data is presented in mg kg^{-1} wet weight (WW), while all bone data is presented as mg kg^{-1} dry weight (DW). When also analysing livers from Doñana birds, Hernández et al. (1999) used a conversion factor of 3.46 to convert dry, to WW liver concentrations. This factor could also be used on the data provided herein for comparison, (i.e., multiply WW concentrations given by 3.46 to determine approximate DW values).

2.3. Statistics and quality control

Statistical analyses were conducted using Minitab Version 13.32, on log-transformed data. Values below the limit of detection (LOD) were considered as one-half of the relevant LOD. Differences in metal concentrations between species were studied using one-way ANOVAs. The variation in metal concentrations were analysed using generalised linear models (GLMs); and are expressed as η^2 (sum of squares for the effect, divided by the total corrected sum of squares). Included in the models were species, age (chick, juvenile, or adult), sex, and location (National or Natural Park). Where information was sufficient, interactions between these factors were also studied. Models were run which included both all species, and, just mallard and coot, since relatively few specimens of the other species (gadwall, pochard, and red crested pochard) studied, were available.

Recovery of elements from samples made up using certified 1000 mg L^{-1} stock solutions, and from bone meal (SRM1486) and bovine liver (SRM1577b) certified reference material, is reported for information in Table 1. Detection limits are also shown, and were calculated based on three times the standard deviation of nine blanks ran at intervals during the analysis of samples.

Table 1
Mean percentage recovery of spikes and certified reference materials

	As	Pb	Zn	Se	Cu
Spike recovery % ($n=5$)	93±7%	97±9%	94±5%	82±3%	—
Detection limit (bone, $n=9$)	0.011	0.156	0.50	0.016	0.783
Detection limit (liver, $n=9$)	0.005	0.155	1.55	0.012	0.719
Bone standard value (% recovery, $n=5$)	0.006[a] (nd)	1.335[b] (93±8%)	147[b] (92±2%)	0.13[a] (72±5%)	0.8[a] (nd)
Liver standard value (% recovery, $n=3$)	0.05[a] (49±10%)	0.129[b] (nd)	127[b] (109±12%)	0.73[b] (79±13%)	160[b] (99±11%)

Bone meal standard (SRM1486); bovine liver standard (SRM1577b)—standard/detection limit values given in mg kg^{-1}.
nd—the standard value is less than or equal to the detection limit.
[a]Uncertified value given for information only.
[b]Certified value given for information only.

3. Results and discussion

3.1. Differences between species

Significant differences in metal concentrations between species were observed for all metals in both liver and bone matrices, except in relation to bone Pb and liver Se (see Tables 2 and 3). Species is also found to be the most important factor explaining the variation in concentrations of all metals in both bone and liver matrices, except in relation to bone Pb and Cu, and liver Se and Pb, (see GLM for all species, Table 4).

In relation to bone As, mallards and pochards contained significantly lower amounts of As than the other 3 species studied. In relation to liver As, mallards again contained significantly less As than the other 4

Table 2
Metal levels in the humerus of 6 waterbirds from Doñana

Species/family	n	Geometric mean (range) and 95% CIs of bone DW concentrations (mg kg^{-1})				
		As[a]	Zn[a]	Se[a]	Pb	Cu
Coot (*Fulica Atra*) Rallidae (rails)	58	0.14 (0.02–0.52) 0.12–0.16 (A)	159.0 (109.4–234.3) 153.2–164.9 (B)	0.14 (0.06–0.32) 0.13–0.15 (B)	2.36 (0.36–28.83) 1.83–3.06 (A)	3.34 (2.18–8.92) 3.15–3.54 (A)
Mallard (*Anas platyrhynchos*) ANATIDAE, Anatini (dabbling ducks)	40	0.05 (0.01–1.76) 0.04–0.06 (B)	154.9 (122.2–205.9) 148.2–162.0 (B)	0.19 (0.11–0.85) 0.17–0.21 (A)	2.23 (nd-134.11) 1.34–3.70 (A)	3.04 (2.42–4.27) 2.92–3.16 (B)
Gadwall (*Anas strepera*) ANATIDAE, Anatini (dabbling ducks)	6	0.11 (0.04–0.22) 0.07–0.18 (A)	211.1 (124.9–247.6) 171.2–260.2 (A)	0.18 (0.13–0.27) 0.15–0.22 (A)	2.19 (0.62–4.05) 1.26–3.83 (A)	2.94 (2.48–3.70) 2.63–3.29 (AB)
Pochard (*Aythya ferina*) ANATIDAE, Aythyini (pochards)	11	0.04 (nd-0.14) 0.02–0.06 (B)	193.1 (163.7–220.8) 182.6–204.3 (A)	0.25 (0.08–1.27) 0.15–0.42 (A)	4.47 (0.87–29.66) 1.92–10.41 (A)	3.22 (2.41–4.52) 2.85–3.65 (AB)
Red crested pochard (*Netta rufina*) ANATIDAE, Aythyini (pochards)	9	0.13 (0.05–0.33) 0.08–0.19 (A)	191.3 (149.4–240.0) 171.9–212.9 (A)	0.16 (0.10–0.31) 0.12–0.20 (AB)	4.05 (1.14–8.79) 2.46–6.65 (A)	3.32 (2.81–3.94) 3.06–3.61 (AB)
Purple gallinule (*Porphyrio porphyrio*) Rallidae (rails)	11	0.01 (nd-0.07) 0.01–0.02	139.3 (97.6–178.2) 126.5–153.4	0.16 (0.04–0.33) 0.11–0.23	1.26 (0.27–23.07) 0.66–2.41	2.71 (2.20–4.09) 2.46–2.98

nd—below detection limit (LOD). Values below LOD replaced by half the LOD for statistics.
Groups of data sharing the same letter in a column are not significantly different ($P > 0.05$; using one-way ANOVA).
[a]Highlights the elements found to be significantly affected by the factor 'species' in the GLM for all species shown in Table 4.

Table 3
Metal levels in the livers of 6 waterbirds from Doñana

Species/family	n	Geometric mean (range) and 95% CIs of liver WW concentrations (mg kg^{-1})				
		As[a]	Zn[a]	Se	Pb[a]	Cu[a]
Coot (*Fulica Atra*) Rallidae (rails)	58	0.02 (nd-0.34) 0.01–0.02 (A)	68.1 (33.8–190.2) 61.8–75.0 (B)	0.36 (0.16–0.85) 0.32–0.39 (A)	0.18 (nd-3.80) 0.14–0.23 (B)	29.06 (7.30–103.34) 24.69–34.20 (C)
Mallard (*Anas platyrhynchos*) ANATIDAE, Anatini (dabbling ducks)	40	0.01 (nd-0.06) 0.01–0.01 (B)	66.4 (29.8–156.9) 56.8–77.5 (B)	0.40 (0.15–0.69) 0.36–0.45 (A)	0.21 (nd-2.81) 0.15–0.29 (B)	27.46 (10.86–194.07) 22.24–33.92 (C)
Gadwall (*Anas strepera*) ANATIDAE, Anatini (dabbling ducks)	6	0.03 (0.01–0.07) 0.01–0.05 (A)	138.9 (79.9–214.8) 102.3–188.4 (A)	0.38 (0.30–0.59) 0.32–0.47 (A)	0.52 (0.31–0.78) 0.40–0.68 (A)	30.12 (17.06–76.14) 19.55–46.39 (BC)
Pochard (*Aythya ferina*) ANATIDAE, Aythyini (pochards)	11	0.02 (nd-0.05) 0.01–0.02 (A)	103.7 (41.0–216.4) 68.3–157.5 (A)	0.38 (0.23–0.53) 0.33–0.45 (A)	0.26 (nd-2.66) 0.12–0.60 (AB)	101.40 (13.82–742.96) 40.64–253.04 (AB)
Red crested pochard (*Netta rufina*) ANATIDAE, Aythyini (pochards)	9	0.02 (0.01–0.04) 0.02–0.03 (A)	79.4 (41.7–220.1) 52.5–120.1 (AB)	0.34 (0.20–0.67) 0.26–0.44 (A)	0.20 (nd-0.88) 0.10–0.38 (B)	134.51 (17.14–639.52) 48.61–372.22 (A)
Purple gallinule (*Porphyrio porphyrio*) Rallidae (rails)	11	0.01 (nd-0.12) 0.01–0.02	53.5 (34.8–114.5) 44.9–63.9	0.18 (0.07–0.49) 0.13–0.25	0.14 (nd-0.77) 0.09–0.23	8.38 (4.77–14.52) 6.96–10.09

nd—below detection limit (LOD). Values below LOD replaced by half the LOD for statistics.
Groups of data sharing the same letter in a column are not significantly different ($P > 0.05$; using one-way ANOVA).
[a]Highlights the elements found to be significantly affected by the factor 'species' in the GLM for all species shown in Table 4.

Table 4
Variance explained (η^2 values) by different factors on metal concentrations in the humerus and liver of waterbirds from Doñana

All species ($n = 5$)	Bone					Liver				
	As	Zn	Se	Pb	Cu	As	Zn	Se	Pb	Cu
Species[a,b]	0.417***	0.299**	0.193**	NS	NS	0.150*	0.147***	NS	0.058*	0.319***
Location[a,b]	NS	0.026*	NS	0.039*	0.078**	0.050**	NS	NS	NS	0.075***
Age[a,b]	NS	0.052*	NS	NS	NS	NS	NS	NS	0.073**	0.032*
Sex[a,b]	NS	NS	NS	NS	NS	0.030*	0.044*	NS	0.087**	NS
Location × age[b]	NS	NS	NS	NS	NS	NS	0.058*	NS	NS	0.036*
Species × location[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.045**
Age × sex[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.027*
Mallards and coots										
Species[a,b]	0.403***	NS	0.187***	NS	0.055*	0.118**	NS	NS	NS	NS
Location[a,b]	NS	NS	NS	NS	0.101**	0.093**	NS	NS	NS	0.058*
Age[a,b]	NS	0.088*	NS	NS	NS	NS	NS	NS	NS	NS
Sex[a,b]	NS	NS	NS	NS	NS	0.034*	NS	NS	0.085**	NS
Species × sex[b]	NS	NS	NS	NS	NS	0.018*	NS	NS	NS	NS
Species × location[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.002*
Age × sex[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.087*

*$P<0.05$; **$P<0.01$; ***$P<0.001$. NS—not significant.
Results for 2 models are shown. Firstly, a model including the 4 factors, species, location, age and sex.[a] Secondly, a model which also includes the interaction terms species × location, species × sex, location × age, location × sex and age × sex[b]. Only significant interaction terms are shown.

species. Gadwalls contained the highest liver As, a finding also noted in relation to blood in Benito et al. (1999). Herein, mallard livers were found to contain a geometric mean As level of 0.01 mg kg^{-1} WW considering all birds, and 0.03 mg kg^{-1} WW considering only Natural Park birds (approximately 0.03 and 0.10 mg kg^{-1} DW). Hernández et al. (1999) presented a much higher arithmetic mean of 0.23 mg kg^{-1} DW ($n = 10$) for mallard livers sampled from Veta Hornitos (considered as National Park herein) 72–89 days after the mine spill. This difference in As concentrations may in part, be related to differences in analytical/statistical methodology.

In terms of Zn concentrations, coot and mallard generally contained lower levels in both bones and livers, than did the other 3 species studied. Again, Hernández et al. (1999) presented an arithmetic mean mallard liver Zn level of 163.0 mg kg^{-1} DW ($n = 10$) and in coots, 288.6 mg kg^{-1} DW ($n = 24$). The DW concentrations presented herein are comparable, at approximately 236 mg kg^{-1} ($n = 58$) for coots, and 230 mg kg^{-1} ($n = 40$) for mallards. Gadwall were found to have the highest mean liver Zn, a finding also made in Benito et al. (1999) in relation to blood metal concentrations.

Coots (and to a lesser extent, red crested pochards) contained relatively low levels of bone Se; and gadwalls had relatively high levels of liver Pb compared to the other 4 species. Benito et al. (1999) also found that gadwall had relatively high blood Pb levels after the spill, second only to mallard in a study of 11 species. Hernández et al. (1999) presented an arithmetic mean gadwall Pb liver level of 3.788 mg kg^{-1} DW ($n = 17$), whilst this paper gives a lower approximate geometric mean DW level of 1.8 mg kg^{-1} ($n = 6$).

In terms of liver Cu, pochards and red crested pochards contained markedly higher levels than the other 3 species studied. The levels in these 2 Aythyini species are up to a maximum of 743 and 640 mg kg^{-1} WW, respectively. Taking into account moisture content, these values represent approximately 2571 and 2214 mg kg^{-1} DW. Although elevated Cu concentrations in these 2 species have been noted before (Mateo and Guitart, 2003; Parslow et al., 1982) they were only noted up to a maximum of 599 and 603 mg kg^{-1} DW in these studies. The reason why these particular species seem to commonly contain such high Cu levels in their livers currently remains unclear. Hernández et al. (1999) presented a mean pochard Cu liver level of 478.5 mg kg^{-1} DW ($n = 11$, range = 52.11–1298); herein the mean pochard liver value in DW is approximately 351 mg kg^{-1}, again lower.

3.2. Differences in relation to location, sex and age

Tables 5 and 6 present and compare the geometric mean and 95% CI concentrations for the metals studied, in relation to location, age and sex.

Whether birds were found dead in the Natural or National Park areas would seem to be an important factor in relation to the concentration of bone Zn, Pb

Table 5
Geometric mean concentration and 95% CIs for 5 metals in 5 waterbird species from Doñana, in relation to location and sex

	Bone			Liver		
	National Pk (99)	Natural Pk (25)	*P*	National Pk (99)	Natural Pk (25)	*P*
As	0.08 (0.07–0.09)	0.11 (0.08–0.16)	NS	0.01 (0.01–0.01)[a]	0.02 (0.02–0.03)[a]	<0.01
Zn	161.0 (155.7–166.5)[a]	180.6 (169.7–192.1)[a]	<0.01	70.2 (64.0–77.0)	87.3 (69.1–110.2)	NS
Se	0.16 (0.15–0.18)	0.18 (0.15–0.23)	NS	0.37 (0.34–0.40)	0.38 (0.34–0.42)	NS
Pb	2.19 (1.71–2.80)[a]	4.60 (2.99–7.08)[a]	<0.01	0.20 (0.16–0.24)	0.26 (0.17–0.38)	NS
Cu	3.12 (3.03–3.20)[a]	3.60 (3.19–4.06)[a]	0.001	28.28 (24.61–32.49)[a]	80.22 (48.55–132.57)[a]	<0.001
	Female (35)	Male (86)		Female (35)	Male (86)	
As	0.08 (0.06–0.10)	0.09 (0.07–0.11)	NS	0.02 (0.01–0.03)[a]	0.01 (0.01–0.01)[a]	<0.05
Zn	167.3 (156.9–178.4)	163.8 (158.2–169.6)	NS	87.7 (72.4–106.1)[a]	68.8 (62.5–75.7)[a]	<0.05
Se	0.16 (0.15–0.18)	0.17 (0.15–0.18)	NS	0.39 (0.35–0.43)	0.37 (0.34–0.40)	NS
Pb	3.39 (2.40–4.78)	2.30 (1.73–3.04)	NS	0.34 (0.23–0.49)[a]	0.17 (0.14–0.21)[a]	0.001
Cu	3.15 (3.01–3.29)	3.24 (3.10–3.39)	NS	43.40 (31.29–60.22)	32.92 (26.98–40.17)	NS

'*n*' values in brackets after location/sex. '*P*' values given for one way ANOVA, comparing metal levels between 'location' and 'sex'.

[a]Highlights the concentration data found to be significantly affected by the factors 'sex' and 'location' in the GLM for all species (excluding interaction terms) shown in Table 4.

Table 6
Geometric mean concentration and 95% CIs for 5 metals in 5 waterbird species from Doñana, in relation to age

	Chick (32)	Juvenile (56)	Adult (36)	'C'–'J'–'A'	'C + J'–'A'
Bone					
As	0.10 (0.08–0.12)	0.11 (0.09–0.13)	0.05 (0.03–0.07)	<0.001	<0.001
Zn	189.6 (177.9–201.9)[a]	160.0 (154.6–165.6)[a]	152.4 (145.0–160.2)[a]	<0.001	0.001
Se	0.19 (0.16–0.23)	0.15 (0.14–0.16)	0.18 (0.15–0.21)	<0.05	NS
Pb	2.46 (1.90–3.19)	2.19 (1.60–2.98)	3.31 (1.93–5.67)	NS	NS
Cu	3.27 (3.03–3.54)	3.22 (3.06–3.39)	3.13 (2.98–3.30)	NS	NS
Liver					
As	0.02 (0.01–0.02)	0.01 (0.01–0.02)	0.01 (0.01–0.02)	NS	NS
Zn	76.5 (62.5–93.7)	71.1 (63.2–79.9)	74.7 (62.9–88.7)	NS	NS
Se	0.39 (0.36–0.43)	0.36 (0.33–0.40)	0.38 (0.33–0.43)	NS	NS
Pb	0.18 (0.13–0.24)[a]	0.19 (0.14–0.24)[a]	0.29 (0.20–0.42)[a]	NS	<0.05
Cu	43.17 (29.05–64.16)[a]	32.09 (25.15–40.96)[a]	33.90 (26.09–44.06)[a]	NS	NS

'*n*' values in brackets after age. '*P*' values given for one way ANOVA, comparing metal levels between 'age'; firstly, comparing chicks, juveniles and adults; secondly, adults against chicks and juveniles combined.

[a]Highlights the concentration data found to be significantly affected by the factor 'age' in the GLM for all species (excluding interaction terms) shown in Table 4.

and Cu, and liver As and Cu (also see GLM for all species in Table 4). In all cases, the mean levels found in birds which died in the Natural Park were higher than those found in the National Park. As noted above, the majority of samples from the National Park were taken in locations at least 5 km from spill affected areas, whilst those taken in the Natural Park were either in or within 2 km of affected areas. In contrast to this finding, in livers, Hernández et al. (1999) actually noted an increase in As with distance from the spill. The elevated liver Cu and As data (liver concentrations may be more representative of short-medium term exposure) may suggest that birds which died in the Natural Park areas sampled herein, 2–3 months after the spill, were indeed exposed to elevated levels of As and Cu in their environment. This exposure may well have been due to a recent event, such as the Aznalcóllar mine spill. However, the elevated bone Zn, Pb and Cu might suggest that, for these 3 elements at least, exposure had in fact been a longer term issue for birds living within the (less well protected and pristine) Natural Park areas. The GLM for all species also shows that location is the most important factor of those studied, in determining bone Pb and Cu levels, and a secondary factor in relation to bone Zn, and liver As and Cu. In relation to liver Cu, location also shows a significant interaction with both 'species' and 'age'. Interaction plots (Fig. 2(a) and (b)) for the factors 'location' and 'age' and 'location' and 'species' clearly show that all age classes and all species studied show elevated liver Cu in the

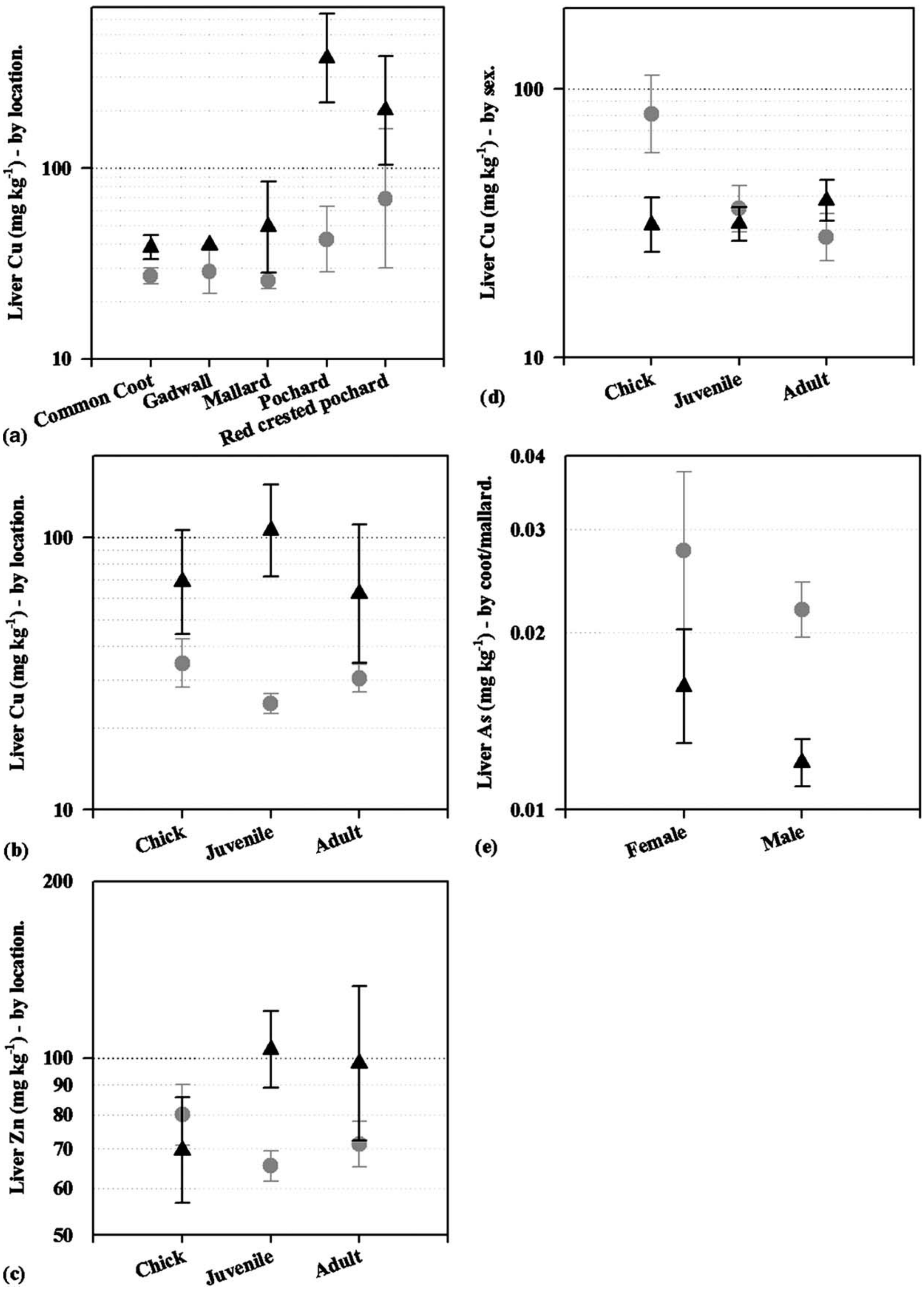

Fig. 2. Metal levels in livers in relation to species, location, sex and age class. Graphs show geometric means and standard error bars. In graphs (a)–(c), black triangles represent Natural Park, grey circles represent National Park. In graph (d), grey circles represent females and black triangles, males. In (e), grey circles are coots, while black triangles are mallards.

Natural Park as compared to the National Park. The GLM for all species also shows a significant interaction between 'age' and 'location' for liver Zn. The interaction plot (Fig. 2(c)) shows that whilst adults and juveniles show elevated liver Zn in the Natural Park areas as compared to the National Park areas, chicks show very similar levels in both areas. This may be related to mobility, in that juveniles and adults may travel further (and therefore into contaminated areas) whilst chicks remain closer to the nest site and are therefore less exposed to wider environmental contamination such as that caused by the mine spill.

In terms of sex, Table 5 shows that female liver As, Zn and Pb appears to be elevated in comparison to that of male birds; the GLM for all species shows that sex is a secondary factor in relation to liver As and Zn, but perhaps the most important factor in relation to liver Pb. In contrast, Mateo and Guitart (2003) found higher liver Pb in males than in females when studying 364 water-birds, from 13 species from Spain; and Benito et al. (1999) predicted that blood lead in females would be lower than in males. There are no apparent differences noted in terms of bone metal concentrations. Liver Cu also shows a significant interaction between 'age' and 'sex' in both the model for all species, and the GLM for coots and mallards alone. An interaction plot (Fig. 2(d)) shows that whilst juveniles and adults show little variation with sex, chicks show a much greater variation, and that female chicks appear to contain high levels of liver Cu in comparison to male chicks. The GLM for mallards and coots shows a significant interaction between 'species' and 'sex' in relation to liver As, and the interaction plot (Fig. 2(e)) shows that mallard males and females contain less liver As than both coot males and females, and that in both species, males consistently had lower liver As levels than did females.

There is a significant difference in different age birds in relation to bone As and Zn (and Se when comparing all 3 age classes), and liver Pb when comparing chicks and juveniles combined with adults (see Table 6). The GLM for all species suggests age is a secondary factor in determining bone Zn, and liver Pb and Cu. Kalisinska et al. (2004) has noted that young mallards from 2 Polish wetlands also tended to have higher levels of Zn in their bones than did adults; but conversely, found higher liver Pb in juvenile birds than in adults. Mateo et al. (2001b) presented data on marbled teal from Spanish wetlands, and, as in this study, found higher liver Pb concentrations in adults than in ducklings. In livers, we did not note an increase in concentration with age for Cu and Zn, as previously reported in Mateo and Guitart (2003).

3.3. Levels in relation to toxicity

Widely accepted guideline values for metal concentrations in waterfowl, in these matrices, do not exist for all the metals studied herein. Pb is perhaps the only metal studied for which such guidelines exist. Background Pb concentrations in liver are considered to be $<2\,mg\,kg^{-1}$ WW, and in bone, $<10\,mg\,kg^{-1}$ DW (Pain, 1986). Only 3 of the 124 specimens studied had liver Pb $>2\,mg\,kg^{-1}$ WW, (2.66–3.80 $mg\,kg^{-1}$), and 2 of these were adult females (one coot, one mallard) from unaffected National Park areas. Sixteen of the 124 specimens had $>10\,mg\,kg^{-1}$ Pb in their bones; 5 coots, 7 mallards and 4 pochards. All but one were juvenile or adult birds, 11 were male and only 4 were from Natural Park areas. Fifteen had concentrations of 10.04–42.46 $mg\,kg^{-1}$, and one male adult mallard had 134.11 $mg\,kg^{-1}$. Since bone Pb tends to accumulate with age and represents long term Pb exposure, it seems unlikely (taking into account the liver data especially) that Pb from the spill was adversely affecting the health of the waterfowl species studied herein, in Doñana, 2–3 months after the disaster. Hernández et al. (1999) detected n.d-24.28 $mg\,kg^{-1}$ DW liver Pb and again concluded that toxic effects were unlikely, and that Pb had not entered the food chain. It is important to note that one of the main sources of Pb in waterfowl in Doñana is Pb shot. In greylag geese found dead in Doñana in 1994–1995, 20% were found to have died of Pb poisoning from Pb shot, (Mateo et al., 1998).

Heinz (1986) suggests that $3\,mg\,kg^{-1}$ WW liver Se may impair reproduction, and that $10\,mg\,kg^{-1}$ may be considered possibly harmful to birds. Since the maximum liver Se reported here is 0.85 $mg\,kg^{-1}$ WW, again, Se levels are below that which would cause concern in the birds studied. A maximum bone concentration of 1.27 $mg\,kg^{-1}$ DW is also considered to be low. Se was, after all, only a minor constituent in the pollution caused by the Aznalcóllar spill. Generally, Se bone concentrations are in the range of 0.04–0.38 $mg\,kg^{-1}$ DW with 3 outlier specimens between 0.85 and 1.27 $mg\,kg^{-1}$ DW. All of these are male birds, 2 are pochard chicks and 1 is an adult mallard; one of the chicks is from the Natural Park. Se liver concentrations are generally between 0.07 and 0.69 $mg\,kg^{-1}$ WW with one higher male coot chick from the National Park at 0.85 $mg\,kg^{-1}$ WW.

No widely accepted guidelines exist in relation to Cu (an essential element) in waterfowl tissues, and a wide variation, as found here, can often be seen between species (Mateo and Guitart, 2003; Parslow et al., 1982). Acute Cu poisoning has been described in Canada Geese (*Branta canadensis*), where liver concentrations were only 187–323 $mg\,kg^{-1}$ DW (Henderson and Winterfield, 1975). Herein, we find 12 of 124 specimens with $>100\,mg\,kg^{-1}$ WW ($>346\,mg\,kg^{-1}$ DW), and 8 of the highest 9 are from Natural Park areas, and are pochards. Mateo and Guitart (2003) reported Cu in waterfowl from 5 Spanish wetlands (including different specimens from Doñana) and 13 species, and determined a liver range of 0.84–599 mg kg^{-1} DW. In 12 specimens from Doñana the range was 17–349 mg kg^{-1} DW in 6 mallards and 6 greylag geese sampled before the Aznalcóllar spill. The highest mallard liver Cu value found in this study (in a juvenile male from the Natural Park) was 194.07 mg kg^{-1} WW (671.48 mg kg^{-1} DW), somewhat high in comparison to the data reported by Mateo and Guitart (2003). Hernández et al. (1999) found 2.054–1298 mg kg^{-1} DW Cu in livers from Doñana. Bone Cu levels are at maximum, 8.92 mg kg^{-1} in this study, and are therefore considered to be low. Although very large differences between species clearly exist in relation to Cu in waterfowl livers, the fact that very high levels are found here in birds from Natural Park areas may suggest (as also concluded by Hernández et al., 1999) that Cu from the spill had entered the waterfowl food chain in Doñana, 2–3 months after the disaster.

Again, accepted limits for Zn do not exist. Levengood et al. (1999) found clinical signs of Zn poisoning in mallards with liver concentrations of 473–1990 mg kg^{-1} DW; Sileo et al. (2003) diagnosed Zn poisoning in wild waterfowl with liver concentrations of 280–2900 mg kg^{-1} and Hernández et al. (1999) found 2.92–1084 mg kg^{-1} DW in the livers of Doñana birds. In this study, the maximum concentration determined was 220.1 mg kg^{-1} WW (761.6 mg kg^{-1} DW). Only 11 specimens contained >150 mg kg^{-1} WW, only 4 of these were from Natural Park areas, but 3 of those 4 contained the highest liver concentrations determined (214.8–220.1 mg kg^{-1} WW). Two of these were female chicks (one gadwall and one red crested pochard), and one was a male juvenile pochard. This may indicate that Zn was beginning to enter the waterfowl food chain in Doñana, 2–3 months after the disaster. The maximum bone Zn found was 247.6 mg kg^{-1} DW, and of the 19 highest (>200 mg kg^{-1} DW), 14 were chicks, 12 were males, and 7 were from the Natural Park areas (a significant proportion of the 25 specimens studied from this area).

Arsenic levels found in bird bones generally ranged from n.d-0.52 mg kg^{-1} DW with one exceptional outlier containing 1.76 mg kg^{-1} DW. This adult male mallard also contained the highest level of bone Pb found (134.11 mg kg^{-1} DW), and it would seem that this bird is likely to have been suffering, or even died from, the effects of Pb poisoning from ingested lead shot (although none was found in the gizzard). Arsenic tends to be present in Pb shot (Hall and Fisher, 1985) and has been found to be elevated in red kite (*Milvus milvus*) bones, which also contained elevated Pb levels (Mateo et al., 2003). Liver As levels generally ranged from n.d-0.12 mg kg^{-1} WW with one outlier at 0.34 mg kg^{-1} WW. Again, this was in a female, adult coot from the National Park, which also had the highest liver Pb found (3.80 mg kg^{-1} WW). Hernández et al. (1999) reported n.d-5.394 mg kg^{-1} DW liver As in Doñana birds, whilst Geode (1985) considered values of <0.5 mg kg^{-1} WW As in bird tissues to be indicative of background concentrations.

Although metal levels in the majority of birds analysed herein seem to be too low to have directly caused the death of these birds, it should be noted that the majority of birds tested appeared to have died as a result of disease/infection. The possibility therefore remains, that these birds were rendered more susceptible to disease/infection due to an elevated level of exposure over a short time period, to a wide combination of metals at a low level. Without comparing metal levels in birds which died of infection/disease with those that did not, it is impossible to draw any firm conclusions in this regard, however other studies (in porpoises) have found that increased exposure to toxins can apparently increase mortality due to subsequent disease/infection (Jepson et al., 1999; Bennett et al., 2001).

3.4. Purple gallinule data

The data for the purple gallinules has not been included in the overall analysis presented, for the reasons noted above. However, Tables 2 and 3 show the metal levels found in the 11 birds analysed, and, in terms of both liver and bone geometric mean concentrations, these birds consistently (excepting bone Se) had the lowest metal levels found compared to the other species studied, in both matrices. Of these 11 purple gallinules, 8 died before the spill in historically uncontaminated National Park areas (6 of which were chicks), and 3 were sampled after the spill (2 in the year 2000). With only 2 adult samples taken after the spill, and 2 before, it is impossible to draw significant conclusions as to the effects of the spill on this species using the data presented herein. Purple gallinules may, however, be an important indicator species, and are worthy of further study within Doñana. This species is sedentary and resident in the heavily impacted Entremuros area, it is a species of 'European Conservation Concern' due to its rarity (Tucker and Heath, 1994), and it feeds on macrophyte rhizomes (Viellard, 1974). Within the Entremuros, metal levels in macrophytes were found to be elevated after the spill (Meharg et al., 1999; Pain et al., 2003; Taggart et al., 2005). Assuming the data provided herein represents 'background' metal concentrations in this species, future monitoring may be useful in terms of assessing the long-term ecotoxicological significance of this potential food chain transfer pathway in Doñana (i.e., contaminated sediment to macrophyte to bird). The other five species studied here, are generally considered migratory (although a certain, limited number of individuals may stay in the Doñana area, all year round) and may therefore be exposed to the contamination caused by the spill for only limited periods in any 1 year.

Hernández et al. (1999) presented data for 10 purple gallinules sampled between June and November 1998 (after the spill), and gave liver DW arithmetic mean values of 24.23 mg kg^{-1} Cu (range, 5.6–50 mg kg^{-1}), 98.8 mg kg^{-1} Zn (2.9–173 mg kg^{-1}) and 0.125 mg kg^{-1} As (0.156–0.29 mg kg^{-1}). Herein we present approximate geometric mean DW concentrations of 0.03 mg kg^{-1} As, 185 mg kg^{-1} Zn and 29 mg kg^{-1} Cu. The Cu and Zn levels presented here are therefore, if anything, a little higher than those presented for post-spill birds by Hernández et al. (1999), however, the As level is again, much lower.

Recently, Gómez et al. (2004) suggested that of 14 waterfowl species studied in Doñana between April 1998 and May 2000, purple gallinules were one of 4 species noted to have had particularly elevated levels of As, Cd, Cu, Zn and Pb in their tissues. Another of the 4 species noted as having elevated tissue metal levels during this period was *Anser anser* (greylag goose). Notably, both of these species are known to feed widely on below ground (bulbs, rhizomes) macrophyte material within Doñana. Taggart et al. (2005) has further suggested that such material may pose a long-term risk to such species, specifically in relation to As (but also in relation to a range of other metals), as residual As from the Aznalcollar spill accumulates (over time) on the surfaces of such material in association with iron-oxide plaque deposition.

4. Conclusions

When also analysing liver samples taken 2–7 months after the spill, Hernández et al. (1999) concluded that Cu and Zn had entered the Doñana waterfowl food chains, and that Pb, Cd and As had not. Studying blood samples soon after the spill, Benito et al. (1999) concluded that levels of Cd and Pb also appeared to be elevated. Based on the data presented herein, we would suggest that:

1. Pb levels are predominantly below limits which would cause ecotoxicological concern. Where they are elevated, there is no consistent evidence that the source of the Pb is from the spill (rather it is more likely to be due to lead shot). There is evidence that birds from Natural Park areas contain more bone Pb than those from National Park areas, but not more liver Pb, which may be more indicative of shorter term, spill related, exposure. Liver Pb tends to be higher in female and adult birds, the latter being potentially consistent with increased exposure due to increased mobility.
2. Se levels are consistently below levels which might be of concern, and there is no evidence that birds from the Natural Park have higher Se levels than those from the National Park. Se levels in the sludge and water from the spill were quite low compared to the other four metals studied herein, hence it would perhaps have been surprising to have found an effect related to location. Levels in bone and liver seem unaffected by sex, however, bone Se levels are lower in juveniles, than they are in either adults or chicks. As an essential element, this may reflect a physiological requirement at a particular growth stage, rather than an effect caused by the spill.
3. Cu levels are, in a significant number of birds, higher than levels previously noted as toxic in certain species. In general, levels are particularly elevated in the pochards (an issue noted in previously published studies). However, maximum levels are much higher than those previously noted; and there is consistent evidence that birds from Natural Park areas contain higher levels in both bone and liver, than do those from the National Park. This would suggest that increased amounts of Cu had indeed entered the waterfowl food chains in Doñana, 2–3 months after the spill.
4. Zn levels again exceed values which may be of concern in certain species, in a number of individuals studied; and 3 of the 4 highest liver values were noted in birds from the Natural Park. However, location is not consistently a significant factor in determining the liver Zn concentrations. Chicks and juveniles were noted to contain consistently higher levels of Zn in bones than adults; and females had higher liver levels than males, but again, this may be related to physiology, rather than the spill. The data presented therefore provides inconclusive evidence as to whether Zn had entered waterfowl food chains in Doñana, 2–3 months after the spill.
5. Arsenic levels are generally below that which would cause ecotoxicological concern; and where they were very elevated, the origin of the As may be lead shot related, rather than from the spill. However, birds from the Natural Park contained higher levels of As than those from the National Park, in liver matrices. Juveniles and chicks also contained higher bone As than adults, (although this may be related to increased bone growth rates in younger birds). We would suggest that the significant difference in As in relation to location provides some evidence that As had entered waterfowl food chains in Doñana, 2–3 months after the spill.

The lack of detailed historical monitoring information in relation to metal levels in birds from Doñana makes it difficult to be conclusive when discussing the impact of the Aznalcóllar spill on the waterfowl studied herein. Future, long-term monitoring, should enable these data, and that provided in Benito et al. (1999) and Hernández et al. (1999), to be placed into context. The contamination caused by the spill is currently undergoing both fluvial and aerial seasonal redistribution, and only detailed long term monitoring of the habitats and species in the area will allow an accurate understanding of the true effects of this disaster on the ecosystems of Doñana.

Acknowledgments

The authors would like to gratefully acknowledge the financial support given by NERC to M.A. Taggart, and the invaluable help given by Hugues Lefranc, Mª Carmen Medina, Antonio Sánchez, and the Monitoring Group of the Doñana Biological Station in collecting the tissue samples. Thanks also to Raquel Baos for her comments on the manuscript, and Margaret Carlisle for providing the map template.

References

Alastuey, A., García-Sánchez, A., López, F., Querol, X., 1999. Evolution of pyrite mud weathering and mobility of heavy metals in the Guadiamar valley after the Aznalcóllar spill, SW Spain. Sci. Total Environ. 242, 41–55.

Benito, V., Devesa, V., Muñoz, O., Suñer, M.A., Montoro, R., Baos, R., Hiraldo, F., Ferrer, M., Fernández, M., González, M.J., 1999. Trace elements in blood collected from birds feeding in the area around Doñana National Park affected by the toxic spill from the Aznalcóllar mine. Sci. Total Environ. 242, 309–323.

Bennett, P.M., Jepson, P.D., Law, R.J., Jones, B.R., Kuiken, T., Baker, J.R., Rogan, E., Kirkwood, J.K., 2001. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environ. Pollut. 112, 33–40.

Beyer, W.N., 2000. Hazards to wildlife from soil-borne cadmium reconsidered. J. Environ. Quality 29, 1380–1384.

Beyer, W.N., Connor, E.E., Gerould, S., 1994. Estimates of soil ingestion by wildlife. J. Wildlife Manage. 58, 375–382.

Beyer, W.N., Spann, J., Day, D., 1999. Metal and sediment ingestion by dabbling ducks. Sci. Total Environ. 231, 235–239.

Galán, E., González, I., Fernández-Caliani, J.C., 2002. Residual pollution load of soils impacted by the Aznalcóllar (Spain) mining spill after clean-up operations. Sci. Total Environ. 286, 167–179.

Galke, M., 1999. Veta La Palma: Mortandad Estival 1997–1998. Unpublished Report. Junta de Andalucia, Spain.

Garralón, A., Gómez, P., Turrero, M.J., Sánchez, M., Melón, A.M., 1999. The geochemical aspects of toxic waters retained in the Entremuros area (Spain). Sci. Total Environ. 242, 27–40.

Geode, A.A., 1985. Mercury, selenium, arsenic and zinc in waders from the Dutch Wadden Sea. Environ. Pollut. 37, 287–309.

Gómez, G., Baos, R., Gómara, B., Jiménez, B., Benito, V., Montoro, R., Hiraldo, F., González, M.J., 2004. Influence of a mine tailing accident near Doñana National Park (Spain) on heavy metals and arsenic accumulation in 14 species of waterfowl (1998 to 2000). Arch. Environ. Contam. Toxicol. 47, 521–529.

González, M.J., Hernández, L., Rico, C., Baluja, G., 1984. Residues of organochlorine pesticides, polychlorinated biphenyls and heavy metals in the eggs of predatory birds from Doñana National Park (Spain), 1980–1983. J. Environ. Sci. Health 19, 759–772.

Grimalt, J.O., Ferrer, M., Macpherson, E., 1999. The mine tailing accident in Aznalcóllar. Sci. Total Environ. 242, 3–11.

Hall, S.L., Fisher, F.M., 1985. Heavy metal concentration of duck tissues in relation to ingestion of spent shot. Bull. Environ. Contam. Toxicol. 35, 163–172.

Heinz, G.H., 1986. Selenium in birds. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Boca Raton.

Henderson, B.M., Winterfield, R.W., 1975. Acute copper toxicosis in the Canada Goose. Avian Diseases 19, 385–387.

Hernández, L.M., Rico, M.C., González, M.J., Hernan, M.A., Fernández, M.A., 1986. Presence and time trends of organochlorine pollutants and heavy metals in eggs of predatory birds of Spain. J. Field Ornithol. 57, 270–282.

Hernández, L.M., Rico, M.C., González, M.J., Montero, M.C., Fernández, M.A., 1987. Residues of organochlorine chemicals and concentrations of heavy metals in Ciconiforme eggs in relation to diet and habitat. J. Environ. Sci. Health 22, 245–258.

Hernández, L.M., González, M.J., Rico, M.C., Fernández, M.A., Aranda, A., 1988a. Organochlorine and heavy metal residue in Falconiforme and Ciconiforme eggs (Spain). Bull. Environ. Contam. Toxicol. 40, 86–93.

Hernández, L.M., González, M.J., Fernández, M.A., 1988b. Organochlorines and metals in Spanish Imperial Eagle eggs, 1986–1987. Environ. Conserv. 15, 363–364.

Hernández, L.M., Gómara, B., Fernández, M., Jiménez, B., González, M.J., Baos, R., Hiraldo, F., Ferrer, M., Benito, V., Suñer, M.A., Devesa, V., Muñoz, O., Montoro, R., 1999. Accumulation of heavy metals and As in wetland birds in the area around Doñana National Park affected by the Aznalcóllar toxic spill. Sci. Total Environ. 242, 293–308.

Jepson, P.D., Bennett, P.M., Allchin, C.R., Law, R.J., Kuiken, T., Baker, J.R., Rogan, E., Kirkwood, J.K., 1999. Investigating potential associations between chronic exposure to polychlorinated biphenyls and infectious disease mortality in harbour porpoises from England and Wales. Sci. Total Environ. 243–244, 339–348.

Kalisinska, E., Salicki, W., Myslek, P., Kavetska, K.M., Jackowski., A., 2004. Using the Mallard to biomonitor heavy metal contamination of wetlands in north-western Poland. Sci. Total Environ. 320, 145–161.

Levengood, J.M., Sanderson, G.C., Anderson, W.L., Foley, G.L., Skowron, L.M., Brown, P.W., Seets, J.W., 1999. Acute toxicity of ingested zinc shot in game-farm mallards. Illinois Nat. History Survey Bull. 36, 1–36.

Mateo, R., Guitart, R., 2003. Heavy metals in livers of waterbirds from Spain. Arch. Environ. Contam. Toxicol. 44, 398–404.

Mateo, R., Belliure, J., Dolz, J.C., Aguilar Serrano, J.M., Guitart, R., 1998. High prevalence's of lead poisoning in wintering waterfowl in Spain. Arch. Environ. Contam. Toxicol. 35, 342–347.

Mateo, R., Bonet, A., Dolz, J.C., Guitart, R., 2000. Lead shot densities in a site of grit ingestion for greylag geese *Anser anser* in Doñana (Spain). Ecotoxicol. Environ. Restoration 3, 76–80.

Mateo, R., Cadenas, R., Máñez, M., Guitart, R., 2001a. Lead shot ingestion in two raptor species from Doñana, Spain. Ecotoxicol. Environ. Safety 48, 6–10.

Mateo, R., Green, A.J., Jeske, C.W., Urios, V., Gerique, C., 2001b. Lead poisoning in the globally threatened marbled teal and white-headed duck in Spain. Environ. Toxicol. Chem. 20, 2860–2868.

Mateo, R., Taggart, M., Meharg, A.A., 2003. Lead and arsenic in bones of birds of prey from Spain. Environ. Pollut. 126, 107–114.

Meharg, A.A., Osborn, D., Pain, D.J., Sánchez, A., Naveso, M.A., 1999. Contamination of Doñana food-chains after the Aznalcóllar mine disaster. Environ. Pollut. 105, 387–390.

Meharg, A.A., Pain, D.J., Ellam, R.M., Baos, R., Olive, V., Joyson, A., Powell, N., Green, A.J., Hiraldo, F., 2002. Isotopic identification of the sources of lead contamination for white storks (*Ciconia ciconia*) in a marshland ecosystem (Doñana, S.W. Spain). Sci. Total Environ. 300, 81–86.

Pain, D.J., 1986. Lead in waterfowl. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Boca Raton.

Pain, D.J., Sánchez, A., Meharg, A.A., 1998. The Doñana ecological disaster: contamination of a world heritage estuarine marsh ecosystem with acidified pyrite mine waste. Sci. Total Environ. 222, 45–54.

Pain, D.J., Meharg, A., Sinclair, G., Powell, N., Finnie, J., Williams, R., Hilton, G., 2003. Levels of cadmium and zinc in soil and plants following the toxic spill from a pyrite mine, Aznalcóllar, Spain. Ambio 32, 52–57.

Parslow, J.L.F., Thomas, G.J., Williams, T.D., 1982. Heavy metals in the livers of waterfowl from the Ouse Washes, England. Environ. Pollut. 29, 317–327.

Pastor, N., López-Lázaro, M., Tella, J.L., Baos, R., Hiraldo, F., Cortes, F., 2001. Assessment of genotoxic damage by the comet assay in white storks (*Circonia ciconia*) after the Doñana ecological disaster. Mutagenisis 16, 219–223.

Pastor, N., Baos, R., López-Lázaro, M., Jovani, R., Tella, J.L., Hajji, N., Hiraldo, F., Cortes, F., 2004. A 4 year follow-up analysis of genotoxic damage in birds of the Doñana area (south west Spain) in the wake of the 1998 mining waste spill. Mutagenisis 19, 61–65.

Sileo, L., Beyer, W.N., Mateo, R., 2003. Pancreatitis in wild zinc-poisoned waterfowl. Avian Pathol. 32, 655–660.

Taggart, M.A., Carlisle, M., Pain, D.J., Williams, R., Osborn, D., Joyson, A., Meharg, A.A., 2004. The distribution of arsenic in soils affected by the Aznalcóllar mine spill, SW Spain. Sci. Total Environ. 323, 137–152.

Taggart, M.A., Carlisle, M., Pain, D.J., Williams, R., Green, D., Osborn, D., Meharg, A.A., 2005. Arsenic levels in the soils and macrophytes of the 'Entremuros' after the Aznalcóllar mine spill. Environ. Pollut. 133, 129–138.

Tucker, G.M., Heath, M.F., 1994. Birds in Europe: Their Conservation Status. Birdlife International.

Vidal, M., López-Sánchez, J.F., Sastre, J., Jimenez, G., Dagnac, T., Rubio, R., Rauret, G., 1999. Prediction of the impact of the Aznalcóllar toxic spill on the trace element contamination of agricultural soils. Sci. Total Environ. 242, 131–148.

Viellard, J., 1974. The purple gallinule in the marismas of the Guadalquivir. Br. Birds 67, 230–236.