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Isotopic identification of the sources of lead contamination for white storks (*Ciconia ciconia*) in a marshland ecosystem (Doñana, S.W. Spain)

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Abstract

White storks (*Ciconia ciconia*) fed in contaminated waters resulting from the Aznacollar acid mining-sludge spillage into the R. Guadiamar, which feeds the eastern flank of the Guadalquivir marshes (Doñana), S.W. Spain. The sludge was rich in a range of toxic elements, and in organic pollutants such as the aromatic amines. Storks did not exhibit elevated metals in their blood immediately following the accident, but chick blood collected the year following the accident showed genotoxic damage compared to the controls. In this study lead isotope analysis was used to assess if the storks had ingested sludge-derived contaminants. The sludge lead isotope ratio was distinct from that of the Doñana sediments. The stork blood lead isotope ratios exactly matched that of the sludge. It was concluded that the storks had ingested sludge-derived contaminants. A detailed study of the lead contamination along the R. Guadiamar and the R. Guadalquivir (of which the Guadiamar is a tributary) was also conducted to place the white stork colony lead exposure in the context of the spatial contamination of the storks' habitat.

Keywords: Doñana; Lead-isotopes; White storks

1. Introduction

The Guadalquivir marshes (Doñana), S.W. Spain, support globally threatened bird and other animal species, and large tracts of the marshes have been designated an Important Bird Area (Heath et al., 2000), a World Heritage Site, a Ramsar Site and a UNESCO Biosphere Reserve. During April 1998 the Aznacollar mine tailings lagoon failure contaminated the Guadiamar river, which feeds the eastern flank of Doñana, with ∼5 million m^3 of metal rich sludge (Pain et al., 1998; Meharg et al., 1999). The sludge contained 0.8%

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zinc, 0.8% lead, 0.5% arsenic, 0.2% copper, 0.05% antimony, 0.005% thallium, 0.0015% mercury, 0.0025% cadmium and 0.001% selenium (Grimalt et al., 1999). The sludge also had a low pH (c.pH 2), resulting in high mobility and solubility of the contaminating metals (Alastuey et al., 1999; Garralon et al., 1999). These metals are known to be toxic to exposed birds (Beyer et al., 1996). The sludge was also contaminated with aromatic amines (Querol et al., 1999).

The year following the accident, white storks (*Ciconia ciconia*) from a colony close to the area affected by the spillage had an elevated incidence of genetic damage compared to birds from distant colonies (Pastor et al., 2001). The white storks from this colony were observed feeding in contaminated zones immediately after the accident (Pain et al., 1998). However, blood of white storks sampled 16 days after the accident did not show any notable elevation in metal levels (Benito et al., 1999). To investigate the apparent discrepancy between observed genotoxic effects and low blood metal levels we determined stork blood lead isotope ratios. Lead isotopes can be used to source lead exposure in wildlife (Hobson, 1999; Scheuhammer and Templeton, 1998). Stork blood lead isotope ratios were related to ratios of the contaminated sludge and background sediments from the Doñana parks to ascertain where the storks had obtained their lead. To complement the lead isotope study, lead contamination along the affected stretches of the Guadiamar were quantified and mapped.

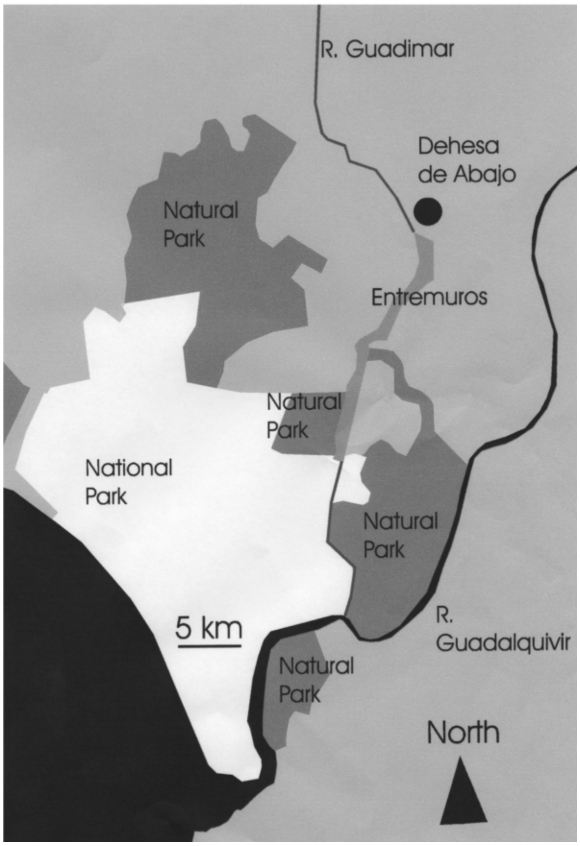

Fig. 1. Map of Doñana showing the designated parks and study sites. The investigated stork colony was at the Dehesa de Abajo.

2. Materials and methods

2.1. Survey of lead contamination

A detailed survey of lead contamination was conducted along the R. Guadiamar during the autumn of 1998. Sediment samples were collected from the dry riverbed for the R. Guadiamar. The area surveyed included the upper zones of the Guadiamar valley that had been directly impacted by the acidified pyrite sludge (Lopez-Pamo et al., 1999), and the Entremuros, a canalised stretch of the Guadiamar river within the Doñana parks system, which was directly metal contaminated by acidified water which preceded the mining sludge down the valley (Garralon et al., 1999) (Fig. 1). Sampling points are illustrated on Fig. 2. Control samples were collected parallel to the Entremuros, 50 m away from the canal bank. The Guadiamar riverbank below the Entremuros was sampled to its confluence with the R. Guadalquivir (Fig. 1). The R. Guadalquivir riverbank was also sampled for comparison as it passes through the highly populated catchment of Seville. Sample locations were recorded on a Global Positioning System (GPS). Sediment to a depth of 10 cm was sampled using a corer. Sediment samples were oven dried and 2 mm sieved. A sub-sample ($\sim$0.2 g) was then digested in 4 ml of Aristar nitric acid on a block digester at 120 °C until the samples went

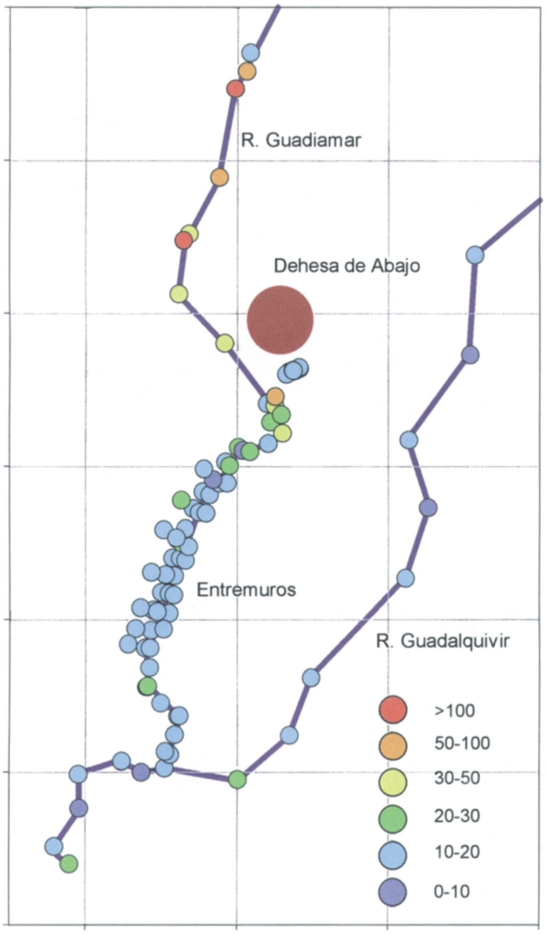

Fig. 2. Lead contamination of R. Guadiamar and Guadilquivir river sediments. The grids on the map are at 10 km intervals. The units of the legend given on the map are mg/kg lead soil dry weight.

clear. The samples, after appropriate dilution, were analysed for lead using flame-atomic absorption spectroscopy and appropriate quality controls.

2.2. Lead isotope ratios

Blood samples were obtained from 10 white stork chicks in June 1999, one breeding season after the accident in 1998, from the colony at Dehesa de Abajo (Figs. 1 and 2), alongside the Entremuros at the southernmost limit reached by the sludge. This is the colony from which birds were reported to have had a high burden of genetic damage.

Sludge from the accident was collected from the impacted areas. Sediment samples were obtained from the Entremuros and from an adjacent non-impacted sites during sampling conducted during September–October (1998) following the accident. Blood, sediments and sludge were digested in nitric acid and hydrogen peroxide. Lead isotope ratios, and total blood lead concentrations, in the digests were then determined by ICP-MS (VG PQII+) as outlined in Farmer et al. (1996). The sediment samples were from the same survey as those used for total lead determinations. Instrumental mass bias is determined daily by measurement of the NIST981 standard. Samples are corrected for mass bias by applying an identical correction to that required to achieve the certified values for NIST981. Subsequently, NIST981 is run every fourth sample as an unknown. Typical precision (2 S.D.) for these bias corrected NIST981 s is $<0.01\%$ within a given analytical session. Isotope ratios are determined in peak jumping mode with 3 points per peak using five 90 s integrations.

3. Results and discussion

Studies on the impact of the mining accident on Doñana have shown that lead levels in the Entremuros were elevated compared to the background levels in the rest of the Doñana National and Natural Parks (Pain et al., 1998; Garralon et al., 1999). Our study shows that this contamination with lead was restricted to the upper reaches of the Entremuros (Fig. 2). The map of lead concentrations along the Guadiamar and Guadalquivir rivers showed that the Guadiamar upstream of the Entremuros is considerably more contaminated than the rest of the river system, with lead levels over 100 mg/kg observed compared to 10–20 mg/kg, typical of the rest of the sites. The very upper reaches of the Entremuros were in places contaminated with levels above 200 mg/kg. The lower reaches of the Guadiamar, below the Entremuros, and the Guadiaquivir are relatively uncontaminated. The findings from our survey are in agreement with other surveys of metal concentrations conducted for the upper reaches of the Gudiamar, above the Entremuros, and for the Entremuros itself (Cabrera et al., 1999; Pain et al., 1998; Vidal et al. 1999; Pain et al., in press). Limited analysis of sediments at the confluence of the Guadiamar and the Guadalquivir, and for the Bay of Cadiz at the mouth of the Guadalquivir, report lead levels of 20 mg/kg (Gomez-Parra et al., 2000), in agreement with the findings of our study (Fig. 2).

Sediments from stretches of the Guadiamar River that pass the stork colony at the Dehesa de Abajo were elevated in lead content (Fig. 2). It is from this stretch, adjacent to contaminated rice and cotton fields (Grimalt et al., 1999) and the upper contaminated parts of the Entremuros, that storks were observed feeding after the accident (Pain et al., 1998). The rice and cotton fields adjacent to the R. Guadiamar were contaminated by the accident. Doñana white storks feed on aquatic vertebrates and invertebrates (Negro et al., 2000). The storks continued to feed on fish and crayfish from these still contaminated stretches long after the accident, and still do so. Thus, even though stork blood lead levels were not high when monitored just after the accident (Benito et al., 1999), the habitat in which they live is lead contaminated, and contaminated with a range of other metals and organic pollutants present in the sludge such as aromatic amines.

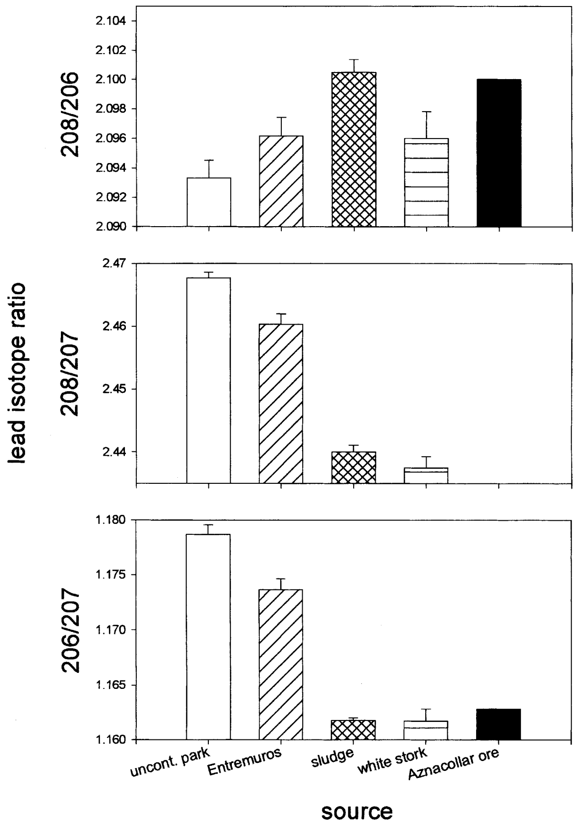

Fig. 3. Lead isotope ratios for bird bloods and Doñana environmental media. The histograms show the mean isotope ratio, and error bars are ±S.E. of the mean. Values for the Aznacollar ore were obtained from Stos-Gale et al. (1995).

3.1. Total blood lead contamination

Blood lead levels in waterfowl are considered elevated at concentrations above 200 ng/ml wet wt., and concentrations above 400 ng/ml are indicative of poisoning (Pain, 1996). The median lead level in the blood of stork chicks analysed was 168 ng/ml ($n=10$), below what is considered as harmful for a range of bird species, although there is not much data on lead toxicity thresholds in Ciconiformes (Franson, 1996). Subsequent analysis of blood for genetic damage, using the Comet assay of chicks from the Dehesa de Abajo colony, at the same time as the bloods taken for lead isotope analysis reported here, showed that this colony population had more DNA aberrations than storks from nearby colonies not impacted by the accident (Pastor et al., 2001). Although blood lead levels in stork are not particularly elevated, and are below threshold levels for lead poisoning, evidence from the blood lead isotope ratios presented below shows that the lead did originate from the spill.

3.2. Lead isotope analysis

Lead isotopic composition was used to reveal if the sources of blood lead contamination could be apportioned. The results showed that the 208:206 ratio did not discriminate well between the samples (Figs. 3 and 4), and ONEWAY ANOVA showed that their was no significant difference in this ratio between uncontaminated parks, the Entremuros,

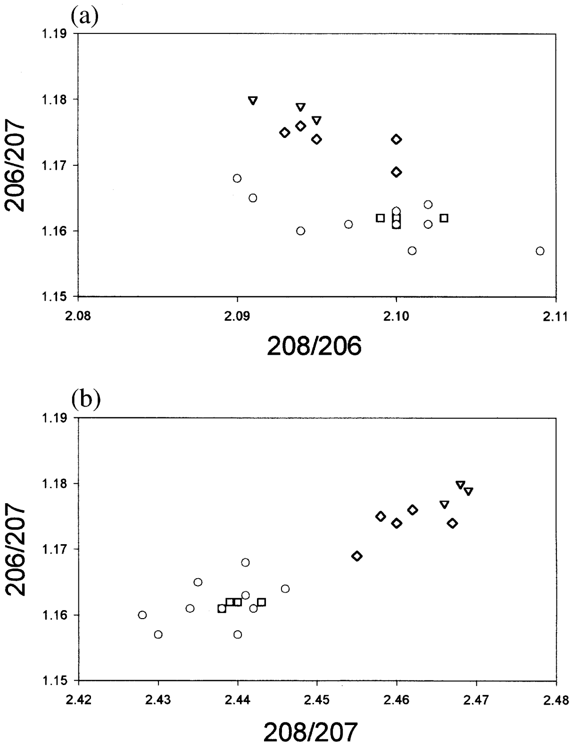

Fig. 4. Scatter plot for lead isotope values of individual samples, from which the means in Fig. 3 were calculated. Storks are represented by the circles, sludge by squares, Entromuros by diamonds and non-impacted Park samples by triangles.

sludge and stork blood samples. The 208:207 and 206:207 ratios, however, were highly indicative of source, with the ANOVA being significant at the $P<0.001$ level for both ratios. The pyrite sludge was very distinct compared to the uncontaminated parks, and identical to Aznacollar ore (Figs. 3 and 4). The Entremuros was directly metal contaminated by acidified water that preceded the sludge down the valley (Fig. 2). The isotope ratios for Entremuros sediment were intermediate between sludge and non-impacted, nearby sediments, though closer to the non-impacted sediments, indicating slight contamination by the accident, in agreement with the total lead survey presented in Fig. 2.

From the plot presented in Fig. 4 it is noteworthy that there was no observable contamination of Doñana samples from leaded petrol, which in W. Europe carries the signature of Australian ore, which has low 208:207 ratios and high 208:206 ratios (Hopper et al., 1991).

Blood from white storks, had lead isotope ratios matching those of the pyrite sludge (Figs. 3 and 4). The stork population at the Dehesa de Abajo is largely resident, and therefore, pollutants present in tissues and bloods are mainly due to local exposure sources. Chicks born one year after the accident have lead isotope ratios identical to the contaminated sludge, and distinct from the background parks signature (Figs. 3 and 4). This lead could have been transferred from the mother to the egg, and/or could have been derived from food collected in the contaminated zones and fed to the chicks. If the storks were foraging in contaminated and uncontaminated zones of the park, it is likely that the lead isotope signature from the sludge will be dominant due to the high lead concentrations found on the sludge. The lead derived from the accident may also be in a more bioavailable form than lead naturally in the sediments.

Blood lead levels, and indeed other metal contaminants, were not particularly high immediately following the accident (Benito et al., 1999), or for lead in chicks born the year following the accident (this study), and lead itself is not considered particularly genotoxic. Lead isotopes are, however, a good marker of the source of contamination to which the storks have been exposed: contaminated sludge, containing many metals and other toxins. The individual and interactive effects of the wide range of toxic elements found in the sludge are not known. Also, the sludge contained the known carcinogens, the aromatic amines (Querol et al., 1999). Levels of these amines have not been reported in bird tissues following the accident. It seems clear that exposure to the accident is causing genotoxic damage to the white stork population. Unpublished data also shows that this population has very high levels of bill and leg deformities, further corroborating the molecular analysis of genotoxic effects (unpublished author's data). The blood lead isotope ratios reported in Figs. 3 and 4 unambiguously show that the dominant lead exposure source to the white storks is lead derived from the Aznacollar accident, obscuring the background parks signature. Thus, the white stork chicks were still being exposed to accident derived toxicants over a year after the spill.

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