



Long-term effects of lead poisoning on bone mineralization in vultures exposed to ammunition sources

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Bone lead accumulation decreases the degree of bone mineralization in vultures exposed to ammunition sources.

ARTICLE INFO

Article history:

Received 20 March 2008

Received in revised form 10 August 2008

Accepted 1 September 2008

Keywords:

Bioaccumulation
Bone mineralization
Egyptian vulture
FTIR
Lead shot

ABSTRACT

Long-lived species are particularly susceptible to bioaccumulation of lead in bone tissues. In this paper we gain insights into the sublethal effects of lead contamination on Egyptian vultures (*Neophron percnopterus*). Our approach was done on the comparison of two populations (Canary Islands and Iberian Peninsula) differing in exposures to the ingestion of lead ammunition. Blood lead levels were higher in the island population (Canary Islands range: 5.10–1780 $\mu\text{g L}^{-1}$ $n = 137$; Iberian Peninsula range: 5.60–217.30 $\mu\text{g L}^{-1}$ $n = 32$) showing clear seasonal trends, peaking during the hunting season. Moreover, males were more susceptible to lead accumulation than females. Bone lead concentration increased with age, reflecting a bioaccumulation effect. The bone composition was significantly altered by this contaminant: the mineralization degree decreased as lead concentration levels increased. These results demonstrate the existence of long-term effects of lead poisoning, which may be of importance in the declines of threatened populations of long-lived species exposed to this contaminant.

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1. Introduction

Lead is a highly toxic heavy metal, which can be released into the environment via numerous routes, but principally by industrial, mining and hunting activities (Fisher et al., 2006). The diffusion into the environment of lead produced by human activities has historically been shown to affect wildlife (Depledge et al., 1993; Eens et al., 1999). Nevertheless, the restrictions implemented over the last few decades have resulted in the current situation where contaminating sources, particularly those from industrial activities in developed countries are very limited. Consequently, the contamination deriving from hunting activities has increased in relative importance in recent decades (Fisher et al., 2006). Ingestion of lead shot and bullet fragments is currently the principal source of lead poisoning for numerous vertebrate species, primarily birds

(Bellrose, 1959; Clark and Scheuhammer, 2003; Mateo, 1998; Pain et al., 2005; Pattee and Hennes, 1983; Redig et al., 1980).

Individual effects of lead poisoning in wild vertebrates have been repeatedly highlighted. Acute poisoning (exposure to a high concentration during a short time period) can lead to death (Mateo, 1998; Ramo et al., 1992), while chronic exposure (prolonged exposure at lower concentrations) can have sublethal effects which affect reproductive success (Burger et al., 1986), behavior (Scheuhammer, 1987), immune response (Redig et al., 1991; Snoeijs et al., 2004) and physiology (Burger, 1995; Fair and Ricklefs, 2002). However, the majority of the studies have limited their scope to descriptive aspects or are based on experimental treatments under controlled laboratory conditions; in consequence, few studies have treated this problem in depth in populations of wild vertebrates (Mateo et al., 1999; Pain et al., 2005).

Bones are a long-term repository for lead, containing approximately 90% of the total body burden in mammals and birds (Ethier et al., 2007). The Pb^{2+} ion readily replaces the Ca^{2+} ion (Ehle, 1993; Scheuhammer, 1987) additionally altering the hormonal regulation of the calcium and the osteoblast function (Pounds et al., 1991; Ronis et al., 2001). This leads to long-term detrimental effects, which have regularly been demonstrated in clinical studies on mammals, and

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fundamentally in humans (Cheng et al., 2001; Latorre et al., 2003; Pounds et al., 1991). Nevertheless, very little information exists on the effects that the substitution of calcium by lead in bone tissue and its consequences in processes such as the mineralization and the alteration of compositional properties and bone mechanics may have (Bjorå et al., 2001; Gruber et al., 1997; Hamilton and O'Flaherty, 1995; Pain et al., 2007). It is foreseeable that these effects could be particularly severe for long-lived vertebrates, whose life-history traits make them prone to lead bioaccumulation over time. Thus, long-term effects of lead poisoning could play an until now unknown role threatening some populations and species of large birds of prey such as the Bald Eagle (*Haliaeetus leucocephalus*), Steller's Sea Eagle (*H. pelagicus*), White-tailed Sea Eagle (*H. albicilla*) and the critically endangered Californian Condor (*Gymnogyps californianus*) which are all known to be affected by lead ammunition sources (Behrens and Brooks, 2000; Church et al., 2006; Franson, 1996; Kenntner et al., 2001; Meretsky et al., 2000).

In this paper we gain insights into the sublethal effects of lead contamination on long-lived vertebrates. We studied the Egyptian Vulture (*Neophron percnopterus*), a small vulture considered as globally endangered (IUCN, 2007) whose most important populations in the Western Palearctic remain in Spain (Donázar, 1993, 2004; Perea et al., 1990). Some Spanish populations of this species have been monitored from the eighties until present, providing an adequate data source for the objectives proposed (Donázar, 1993; Donázar et al., 1996, 2002). Our approach is based on the comparison of two populations, one resident on islands (Canary Islands) and the other a migratory continental one (Iberian Peninsula) which are potentially differentially affected by this contaminant (see Donázar et al., 2002). Our first hypothesis is that the island population, being resident, suffers higher exposure than the migratory continental one to the ingestion of lead shot during the winter hunting season. Consequently we can predict that (a) we should find a higher frequency of lead shot in regurgitated pellets and of acute-type poisoning shown by blood analysis. As a corollary of the former prediction, (b) in the island population we should find a seasonal pattern with higher lead blood levels during the hunting season. Our next hypothesis is that the contamination has long-term effects on individuals. Given that the bone lead level reflects life time exposure, we predict that (c) the Canary Islands Egyptian Vultures will show higher contaminant levels in this tissue, and (d) there will be an effect of bioaccumulation with age. Finally, we expect to find negative effects on the affected individuals, such that (e) a negative relationship will exist between lead concentration and compositional properties of the bone tissue.

2. Materials and methods

2.1. Area and study populations

We analyzed samples of Egyptian Vultures from Fuerteventura, Canary Islands (30 breeding pairs; 1662 Km²) and from the Iberian Peninsula. Within the latter area, individuals from Cádiz (30 b.p.; 9500 Km²), the Ebro valley (100 b.p.; 19,000 Km²) and Segovia (25 b.p.; 5000 km²) were included. Throughout its distribution, Egyptian Vultures usually consume livestock carcasses in "vulture restaurants", but also scavenge heavily on wild prey (mainly rabbit *Oryctolagus cuniculus* and pigeon *Columba* spp. carcasses). In Iberia, garbage dumps also provide an important fraction of their trophic needs (see Donázar, 1993; Donázar et al., 2002). The Iberian populations remain in their distinct breeding areas between March and September and so consequently their presence does not overlap with the hunting season in Spain, which used to begin at the end of the summer, finishing in January–February before the arrival of the transaharan migrants (Cramp and Simmons, 1980; Del Hoyo et al., 1994). In Fuerteventura, the Egyptian Vultures are sedentary, and so are also present on the breeding grounds during the hunting season (August–November).

2.2. Field procedures

To determine the incidence of lead shot ingestion in the mainland population and compare it with that previously observed in the island one (see Donázar et al., 2002), we collected 327 regurgitated pellets at the communal roosts used by the species in the Ebro

valley from 1999 to 2003. We determined the presence of ingested shot by X-rays. Pellets apparently containing shot were subsequently dissected to confirm the presence of lead.

From 1999 to 2005, we obtained 137 blood samples of Egyptian Vultures on the Canary Islands, both from fledglings ($n = 61$) and individuals of other ages trapped during cannon-netting ($n = 76$). In addition, we obtained 32 samples of immature and adult mainland birds (10 individuals in the Ebro valley in 2002, 19 in Segovia in 2003–2004 and three in Cádiz in 2003). The birds' ages were determined using plumage characteristics (Forsman, 1998) as 1 = fledglings, 2 = individuals of 1–2 years old, 3 = 2–3 years, 4 = 3–4 years, 5 = 4–5 years (adults) and 6 = individuals ≥ 5 years old). We extracted a small quantity of blood (1 ml) from each individual from the brachial vein. It was placed in a tube containing lithium-heparin and immediately frozen at -20°C .

We extracted bone tissue samples from 39 Egyptian Vulture carcasses (28 from the Canary Islands and 11 from the Iberian Peninsula), all found dead in the field between 1999 and 2004. The age of 16 of the island birds and all of the continental birds was known. Age and sex was known for 14 island birds and five continental birds. We extracted the humerus of each individual, which was measured and weighed after meticulous clearing of all muscle material with a scalpel (right humerus 66.7% of cases, left humerus 33.3%). The humerus has frequently been used in studies monitoring the exposure of birds to lead (Ethier et al., 2007). As a pneumatic bone with little hemopoietic activity (Schepelmann, 1990), it may be less likely to be affected by acute exposures due to the absence of the bone marrow and as a result more reliably reflect chronic exposure to lead. Moreover, lead concentrations in the humerus are similar to and highly correlated with those of other long bones such as the femur (Mateo et al., 2003). We obtained from each humerus a fragment approximately 2 cm long from the diaphysis. We washed the sections obtained repeatedly in distilled water to remove any possible traces resulting from the dissection and cutting processes. Finally, we grounded the samples with a cryogenic mill (Freezer/Mill, SPEX) for later analysis.

2.3. Laboratory analyses

2.3.1. Recent exposure

We determined the blood lead concentration using atomic absorption spectrophotometry in graphite furnace (GF-AAS) (AAAnalyst 800, Perkin Elmer) following the procedure described in Mateo et al. (1999). Lead concentrations in $\mu\text{g L}^{-1}$ are the result of the average of three replicates. Additionally, we triple processed a bovine blood sample CRM 196 with an average (SD) of 772 (11) $\mu\text{g L}^{-1}$, giving a result of 767 (8) $\mu\text{g L}^{-1}$, with an RSD of 1.04%. In compliance with the rules of use, the result is acceptable as it falls within the value of the average $\pm 2\text{SD}$ (of 95% of the laboratories which have participated in the analysis of the certified sample). The detection limit for lead was 0.5 $\mu\text{g L}^{-1}$. We interpreted blood lead levels as background ($<200 \mu\text{g L}^{-1}$), subclinical (200–1000 $\mu\text{g L}^{-1}$) and toxic levels ($\geq 1000 \mu\text{g L}^{-1}$) as reviewed by Franson (1996) for Falconiformes.

2.3.2. Chronic exposure

We analyzed the lead content in the bones using inductively coupled plasma with optical emission spectrophotometry (ICP-OES, Perkin-Elmer Optima 5100 DV) and GF-AAS. The sample, 50 mg of ground bone, was digested with 1 ml of 70% nitric acid (HNO_3) and 1 ml of 30% nitrogen peroxide (H_2O_2) in a microwave oven and finally diluted with ultrapure water to give a 10 ml sample. Bone lead levels were interpreted as background ($<10 \mu\text{g g}^{-1}$) or abnormal exposure ($\geq 10 \mu\text{g g}^{-1}$) according to the data reviewed by Mateo et al. (2003).

We investigated the effect of lead contamination on bone mineralization by means of Fourier transformed infrared spectrometry (FTIR), normally used to analyse bone chemical composition (Miller et al., 2001; Ou-Yang et al., 2001; Rey et al., 1991). We undertook it by mixing and homogenizing 5 mg of powdered bone sample with 90 mg of KBr. The tablets were prepared under a pressure of 10 metric Tons for 10 min. We registered the infrared spectra on a Magna IR200 Nicolet FTIR spectrometer. The spectra were acquired in the absorption mode between 400 and 4000 cm^{-1} , with a resolution of 2 cm^{-1} and a total of 128 scans per sample. A reference tablet (95 mg of BrK) was used for background correction.

We determined the organic and mineral material content of the bone samples measuring the area of the absorption bands associated with molecular groups of carbonate, phosphate, amide and C–H aliphatic in the infrared spectrum. We undertook the band analyses and deconvolution of peaks using the computer programs EZ-OMNIC[®] and PeakFIT[®]. The deconvolution of overlapping peaks was resolved using a second derivative method. This method allows a more accurate and more detailed quantitative analysis of the spectrum. A detailed description of the methodology employed is described elsewhere (Rodríguez-Navarro et al., 2006). We calculated the degree of mineralization of the bone as:

$$\text{Mineralization degree} = \frac{\text{phosphate mineral} (\nu_3\text{PO}_4\text{-band area } 900\text{--}1200 \text{ cm}^{-1})}{\text{collagen} (\text{amide I - band area } 1660 \text{ cm}^{-1})}$$

2.4. Statistical analyses

Lead concentrations, both in blood and bone samples were not normally distributed (Shapiro-Wilk, $p < 0.001$), so were normalized through logarithmic transformation in both cases.

We compared the percentage of pellets containing lead shot between the island and continental populations by a Chi-test.

We tested differences in blood lead concentrations between the populations through a *T* test for independent samples. We determined the relation between blood lead levels and hunting activity in the island population using a univariate analysis of variance with the lead concentration as the response variable. We included the capture date: during the hunting season (1) or outside it (0); the sex: female (1) or male (2); and the age of the individuals (1–6, see above for definition of age categories) as model factors. The possible interactions were considered in the model.

We analyzed the possible differences in bone lead concentrations between the populations by means of an analysis of the variance (ANOVA) considering all individuals combined. We included population: Canary Islands (1) or Iberian Peninsula (0); the sex: female (1), male (2) and the age (1–6) as factors. Secondly, we undertook a similar ANOVA analysis but only using adult individuals (≥ 5 years). We investigated the existence of a bioaccumulation effect through a univariate analysis of variance, considering the bone lead concentration as the response variable. The factors corresponding to age (1–6) and sex, as well as their possible interactions were considered in the model. We analysed the effect of age on the bone lead concentration only for the island population, given that all the continental individuals of known age, except for one, were adults ($n = 10$). Subsequently, we examined the relationship between bone lead concentrations and age through a Pearson correlation (one-tailed test).

We analysed the relationships between the degree of bone mineralization with the individual and population characteristics through a univariate analysis of variance, including population, sex and age as factors. The lead concentration was included as a covariate. Finally, we analysed the effect of lead on the compositional properties of the bone (degree of mineralization) through a Pearson correlation (one-tailed test).

3. Results

3.1. Lead intoxication in relation to geographical and seasonal patterns of hunting activities

We found no lead shot in the continental pellets examined ($n = 327$), which was significantly different ($\chi^2 = 14.9$, $DF = 1$, $p = 0.01$) from that found in a previous study carried out in the Canary Islands, where 5% of the pellets examined contained lead shot ($n = 424$; Donázar et al., 2002).

Although the mean blood lead concentration was significantly higher in the continental population: *T* test for unequal variances $t = 2.50$, $DF = 66.56$, $p = 0.01$, 95% $CI = 0.03$ – 0.30 (Lavene test of variance equality $F = 5.43$, $p = 0.021$), both populations presented different distributions of blood lead concentrations (Fig. 1). This different distribution stems from the fact that only one individual from the mainland population presented a concentration indicative of abnormal exposure ($217.30 \mu\text{g L}^{-1}$), whereas in the island population 10 individuals (7.30%) showed subclinical levels above $200 \mu\text{g L}^{-1}$, and three of these (2.19%) showed blood lead levels of over $500 \mu\text{g L}^{-1}$. One of these birds, captured in 2000 and which subsequently disappeared, showed a concentration of $1780 \mu\text{g L}^{-1}$, considered to be a toxic level birds of prey (Franson, 1996).

Blood lead levels were significantly higher in the island population during the hunting season (geometric mean = 93.33 , 95% $CI = 70.79$ – 123.03 , $n = 47$) than outside it (geometric mean = 28.84 , 95% $CI = 24.55$ – 34.67 , $n = 90$) ($F_{1,132} = 62.66$, $p < 0.001$), and differed marginally between the sexes ($F_{1,132} = 3.08$, $p = 0.08$) being higher in males (geometric mean = 44.67 , 95% $CI = 33.11$, $n = 57$) than for females (geometric mean = 41.69 , 95% $CI = 33.11$ – 51.29 , $n = 79$). In addition, an interaction was apparent: males during the hunting season showed the highest levels of lead ($F_{1,132} = 8.32$, $p = 0.005$). Age and its possible interactions showed no significant effect ($p > 0.05$ in all cases).

3.2. Lead accumulation in bone and its consequences on mineralization

Only one individual 3 years old from the island population showed bone lead levels $>20 \mu\text{g g}^{-1}$, considered to indicate excessive exposure and absorption of Pb at some stage. Moreover,

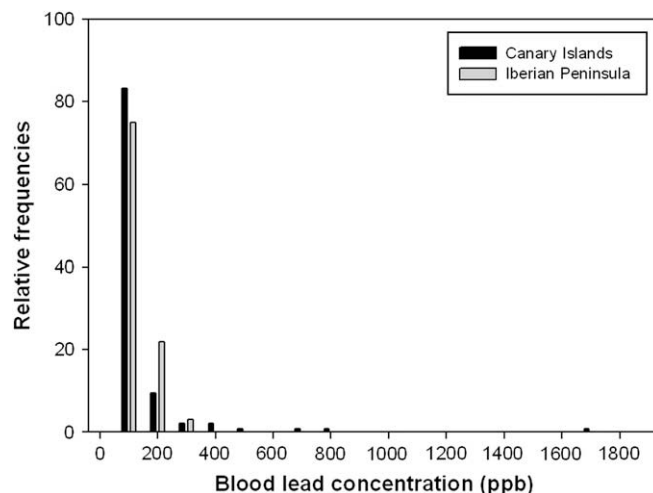


Fig. 1. Distribution of blood lead concentrations (ppb = $\mu\text{g L}^{-1}$) in the mainland ($n = 32$) and the island ($n = 137$) populations of Egyptian Vulture.

Pb concentrations >20 – $30 \mu\text{g g}^{-1}$ are often found in birds that have died of Pb poisoning (Pain et al., 2007).

When all age classes were considered, bone lead levels did not differ significantly between the continent (geometric mean = 6.17 , 95% $CI = 4.27$ – 8.91 , $n = 11$) and the island (geometric mean = 7.42 , 95% $CI = 5.25$ – 8.91 , $n = 28$) (ANOVA, $F_{1,37} = 0.21$, $p = 0.65$). However, bone lead levels increased with age (ANOVA, $F_{5,22} = 4.85$, $p = 0.004$). Considering only adult birds (≥ 5 years), the lead concentration was significantly higher for the island population (geometric mean = 8.13 , 95% $CI = 6.03$ – 10.72 , $n = 11$) than for the mainland one (geometric mean = 5.49 , 95% $CI = 4.07$ – 7.41 , $n = 10$) (ANOVA, $F_{1,19} = 4.5$, $p = 0.04$). Sex showed no significant effect in any of the analyses.

We found a bioaccumulation effect of bone lead with age in the island population (ANOVA $F_{5,11} = 4.418$, $p = 0.019$, $n = 17$) (Fig. 2). The correlation between the variables was positive (Pearson $r = 0.54$, $p = 0.013$, $n = 17$).

Age or sex had no significant effects on the degree of bone mineralization. However, the degree of mineralization was negatively correlated with the bone lead concentration when we combined all data from both populations (Pearson $r = -0.30$,

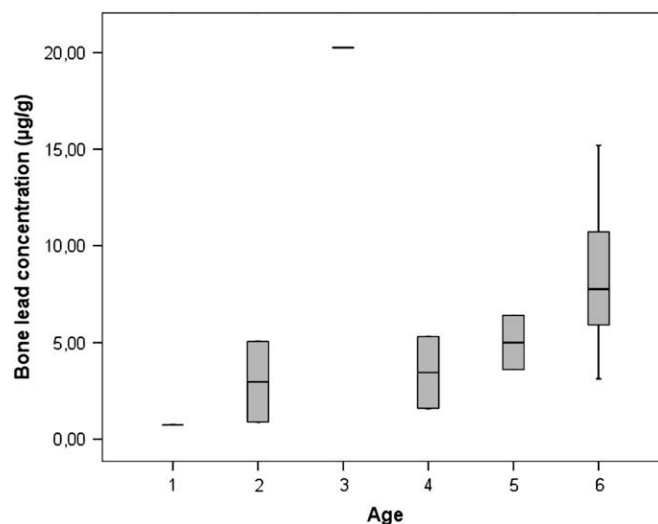


Fig. 2. Bioaccumulation effect of bone lead with age in the island population of Egyptian Vultures.

$p = 0.032$, $n = 39$). This correlation was even stronger when considering only adult birds (Pearson $r = -0.63$, $p = 0.001$, $n = 20$) (Fig. 3).

4. Discussion

Our study demonstrates that ingestion of lead ammunition is related to the accumulation of lead in bones of Egyptian Vultures. It ultimately affected the degree of bone mineralization, this being lowest in those birds with highest bone lead concentrations. Numerous studies indicate that heavy metals, and in particular lead, can affect the correct mineralization of the bone (Gruber et al., 1997; Hamilton and O'Flaherty, 1995), directly or indirectly altering the renewal rate by affecting osteoblasts and osteoclasts activity (Berglund et al., 2000; Carmouche et al., 2005; Kanti and Smith, 1997; Puzas et al., 1992) and playing an important role in the development of bone pathologies (Bjorå et al., 2001; Smits et al., 2005; Puzas, 2000). However, the relationship between bone lead and mineralization has never before been described in wild populations (but see Bjorå et al., 2001), even though it is reasonable to consider it is a logical consequence of repeated lead ingestion and accumulation in long-lived species. The reduction in bone mineralization could mean an increase in bone fragility (Fleming et al., 2000; Whitehead and Fleming, 2000). Indeed, in the island population of Egyptian Vulture an unusually high level of frequency of fractures and even leg amputations has been recorded (8% until 2007, $n = 150$) while this situation has not been detected in the mainland population ($n > 2000$, authors, unpublished data). Although on occasions these fractures are derived from entanglement on ground wire stabilizers (Gangoso and Palacios, 2002), very probably, these fractures may be facilitated by a greater bone fragility (Bjorå et al., 2001; Smits et al., 2005).

The ecology of the island population makes it particularly susceptible to poisoning from ammunition sources. The sedentary nature of the island birds leads to a higher probability of ingestion of lead shot as compared to the mainland population, since they coincide with the hunting season on the island. This appears to be supported given that the blood lead levels found in birds from the Canary Islands were much higher during the hunting season than outside it. The volcanic origin of the island makes the recovery of wounded game such as wild rabbits and Barbary Partridges (*Alectoris barbara*) extremely difficult. In addition, certain introduced

species and of little hunting interest, such as feral pigeons (*Columba livia*) and barbary ground squirrels (*Atlantoxerus getulus*) are habitually shot but not retrieved, with the subsequent risk of them being ingested by scavengers (Knopper et al., 2006). In addition, the frequency of individuals in the island population with blood lead levels indicative of abnormal Pb exposure ($>200 \mu\text{g/L}$; Franson, 1996) was higher than that of mainland birds which are less exposed to the chance of consuming shot animals. However, the mainland populations were not free of the poisoning risk, given the frequency of individuals with blood lead levels of $<200 \mu\text{g L}^{-1}$. Although the Iberian Egyptian vultures are not present in Spain during the main hunting season, they are exposed to short hunting periods which takes place in August–September, as well as the ingestion of hunted game from shot at but not killed or crippled game from the previous hunting season. Besides, as found in other bird species, other localized contaminant sources could be operating, such as industrial activity or the ingestion of contaminated materials in rubbish tips where mainland Egyptian vultures feed regularly (Donázar et al., 1996; Blanco et al., 2004; Smits et al., 2005).

Male Egyptian Vultures were significantly more affected by lead contamination during the hunting season, which could be related to sexual differences in the food searching behavior, a phenomenon described in dimorphic raptors where spatial segregation or food niche partitioning is seen (Hedrick and Temeles, 1989; Lee and Severinghaus, 2004). Indeed, our data indicate that females concentrate on Fuerteventura at predictable food sources (“vulture restaurants”) while males search for more prey but which are less predictable spatially (authors, unpublished data). Likewise, bioaccumulation of lead in animal tissues is a highly sex-linked process, given that the forms of ingesting and excreting the contaminant could differ notably between males and females (Tejedor and González, 1992).

The consequences at the population level from lead poisoning remain to be determined. As noted above, it is reasonable to assume that the probability of bone fracture is increased given alterations in the bone mineralization process, which undoubtedly lowers survival rates and/or at least the effective population size, given that no live individuals with apparent fractures has been bred in the island population studied (authors, unpublished data). The island vultures also have a very low productivity rate (0.5 chicks/pair/year, Donázar et al., 2002) and it seems reasonable to consider that the lead could be playing an important role in this, especially given that the lead accumulated in the bones of the females is mobilized during eggshell formation (Taylor, 1970) and transmitted to both the eggshell and to the embryo (Edens and Garlich, 1983; Grandjean, 1976; Pattee, 1984). Moreover, lead may affect sperm quality and consequently the fertilization rate (Benoff et al., 2000; Castellanos et al., in press). Additionally, it has hypothesized that the accumulation of toxins at subclinical levels could be the cause of immunosuppression, which increases susceptibility to infectious diseases (Blanco et al., 2004; Daszak et al., 2001; Dobson and Fofopoulos, 2001; Snoeijs et al., 2004). In the island Egyptian Vulture population we find they show poor immunocompetence and a comparatively higher susceptibility to pathogen incidence (Gangoso, 2006), which, although it could have its origin in multiple causes, given the results of the contaminants analyses leads us to suspect that some form of interaction between these factors should exist.

Summarizing, our study demonstrates that a population of long-lived vertebrates, in this case an avian scavenger, exposed to the ingestion of lead shot can be affected over the long-term by the bioaccumulation of this heavy metal in the bone tissue through a loss of mineralization. The ultimate consequences of poisoning by lead can be readily underestimated given that the sublethal effects are difficult to detect and may only show over a very long time

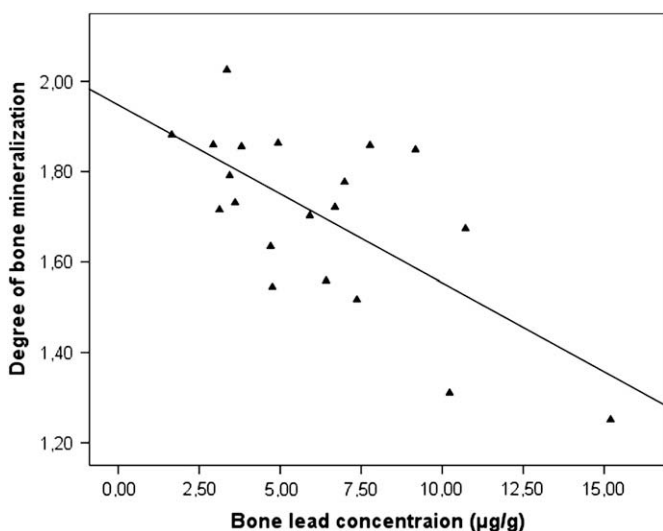


Fig. 3. Negative relation between bone lead ($\mu\text{g g}^{-1}$) and degree of bone mineralization in adult Egyptian Vultures (≥ 5 years old) from the island and mainland populations (fit line $\text{Sq r lineal} = 0.45$ is showed).

period and in combination with other factors (Franson et al., 1983; Mateo et al., 1997; Rocke and Samuel, 1991; Smits et al., 2007). Regular monitoring based on blood analyses from regular field sampling is consequently important to reveal recent exposure to lead, but provides no information regarding the duration of exposure, and, if it doesn't cause the death of the individual, even less information on the long-term effects on the organism (Fry, 2003). Without doubt, the hidden effects such as those revealed in this study, may be acting as the limiting factors in relation to the decline and recovery of populations of long-lived vertebrates exposed to lead poisoning (Fry, 2003; Pattee et al., 1990).

5. Conclusions

We analyzed the long-term effects of lead poisoning in two populations of Egyptian vultures (insular vs. mainland). The ecological context of the insular population determined an elevated exposition to lead shot, and consequently, higher levels of blood and bone lead than the mainland one. Moreover, blood lead levels were higher during the hunting season in the island population, especially for males. In addition, we found a clear effect of bioaccumulation of bone lead with age. The main conclusion drawn is that lead causes a reduction in bone mineralization. These hidden effects of lead poisoning may be of importance in the declines of threatened populations of long-lived species exposed to this contaminant.

Acknowledgements

We would like to thank A. Trujillano and M. de la Riva for their helpful assistance during the fieldwork. The Consejería de Medio Ambiente del Cabildo Insular de Fuerteventura, the Viceconsejería de Medio Ambiente del Gobierno de Canarias, and the Projects REN 2000-1556 GLO and CGL2004-00270/BOS funded this research. We also thank financial support from the Spanish government through grant REN2003-07375.

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