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Lead and arsenic in bones of birds of prey from Spain

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“Capsule”: *Raptors feeding on species targeted by hunters in upland habitats suffer from lead poisoning.*

Abstract

The bones (humerus and/or femur) of 229 birds of prey from 11 species were analyzed for Pb and As to evaluate their exposure to Pb shot. The species with the highest mean Pb levels were red kite (*Milvus milvus*) and Eurasian griffon (*Gyps fulvus*), and the species with the lowest levels were Eurasian buzzard (*Buteo buteo*) and booted eagle (*Hieraetus pennatus*). Red kite also had the highest mean As level, an element present in small amounts in Pb shot. Elevated bone Pb concentrations (> 10 µg/g dry weight) were found in 10 birds from six species. Clinical signs compatible with lethal Pb poisoning and/or excessive bone Pb concentrations (> 20 µg/g) were observed in one Eurasian eagle-owl (*Bubo bubo*), one red kite, and one Eurasian griffon. Pb poisoning has been diagnosed in eight upland raptor species in Spain to date.

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Keywords: Lead poisoning; Falconiformes; Strigiformes; hunting; Arsenic

1. Introduction

Lead (Pb) poisoning in raptors is usually caused by the ingestion of prey with Pb shot embedded in their flesh (Scheuhammer and Norris, 1996). This situation can be common in raptor species inhabiting areas with high shooting pressure, such as wetlands. Pb shot exposure is important in Eurasian marsh-harrier (*Circus aeruginosus*) in the wetlands of France (Pain et al., 1993, 1997) and Spain (Mateo et al., 1999), and in Spanish imperial eagle (*Aquila adalberti*) and red kite (*Milvus milvus*) in the Guadalquivir marshes of southwestern Spain (Mateo et al., 2001a). However, some upland areas are also intensively used for pigeon, partridge and rabbit hunting in Spain, and cases of Pb poisoning have been described in several species of birds of prey in upland habitats, including golden eagle (*A. chrysaetos*) (Cerradelo et al., 1992), Spanish imperial eagle (Hernández, 1995), Eurasian griffon (*Gyps fulvus*) (Mateo et al., 1997), Eurasian buzzard (*Buteo buteo*), common barn-owl (*Tyto alba*) and Northern long-eared owl (*Asio*

otus) (Guitart et al., 1999). Although the effect of the mortality caused by Pb poisoning on Spanish raptor populations is not yet known, this has been relevant in the decline (Wiemeyer et al., 1988), and successful reintroduction of California condor (*Gymnogyps californicus*) in the USA (Behrens and Brooks, 2000). Pb poisoning is an important threat for the endangered Steller's sea eagle (*Haliaeetus pelagicus*) and white tailed sea eagle (*H. albicilla*) in the Palearctic (Kim et al., 1999; Kenntner et al., 2001). Their nearctic relative, the bald eagle (*H. leucocephalus*), still suffered from Pb poisoning even after the USA Pb shot ban on waterfowl hunting in 1991 (Kramer and Redig, 1997).

Capture of scarce and protected species for sample collection, such as birds of prey can be difficult and is not free of risk for valuable specimens. A non-invasive method such as the collection of regurgitated pellets can be used to study the presence of ingested Pb shot and this gives information on the exposure frequency, seasonality and the food items associated with Pb shot ingestion (Mateo et al., 1999, 2001a). However, pellet collection can be difficult to accomplish in raptors from wooded or mountainous areas, especially out of the breeding season, when Pb shot ingestion by the consumption of shot prey is more common because of the

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overlap with the hunting season (Mateo et al., 1999, 2001a). Between 1990 and 2000, 21,181 birds from 40 species of Falconiformes and Strigiformes were admitted to 23 wildlife rehabilitation centers in Spain (Mañosa, 2002), and although some bias may be expected, those animals that died can provide a useful sample for contaminant monitoring. Studies to assess Pb exposure in raptors have been undertaken by means of liver analyses of birds sampled by this method (Pain and Amiard-Triquet, 1993; Pain et al., 1995). Soft tissues are useful because they tend to reflect recent Pb exposure (Pain, 1996). However, bone tissue can also be useful when rates of Pb shot ingestion are expected to be low, because bone Pb level reflects chronic exposure and contains 84–90% of total Pb body burden in raptors (García-Fernández et al., 1997). Moreover, birds collected out of the hunting season can also be useful because Pb bone levels in birds tend to remain elevated for more than 30 weeks after Pb shot ingestion (Stendell et al., 1979). Bones have been successfully used in large-scale studies to assess Pb exposure in waterfowl and American woodcock (*Scolopax minor*) (Stendell et al., 1979; Wickson et al., 1992; Pain et al., 1992; Scheuhammer and Dickson, 1996; Scheuhammer et al., 1999).

This paper presents bone Pb concentrations found in medium–large sized birds of prey from Spain. The relationship between Pb exposure and the diet of the species is discussed, and the species that may require further monitoring are highlighted. Arsenic (As) concentrations were also determined because this element is present in small amounts in Pb shot (0.23–0.84%; Hall and Fisher, 1985a).

2. Materials and methods

Bones of raptors were acquired between 1998 and 2001 from six wildlife rehabilitation centers located in the east (Barcelona, Lleida, Tarragona, Valencia) and center of Spain (Madrid and Toledo). The species requested were those that fed on prey normally targeted by hunters in upland habitats (ungulates, rabbit, hare, partridge and pigeon). Depending on the center either humerus ($n=90$), femur ($n=47$) or both bones ($n=92$) were collected from 229 birds of 11 species. Bones without fractures were collected with the adjacent muscle, frozen at $-20\text{ }^{\circ}\text{C}$ and submitted with clinical data, sex, age, location and the date of collection. The causes of death reported by the wildlife rehabilitation centers were electrocution (20.5%), traumatism (20.5%), illegal shooting (15.7%), illegal poisoning (6.1%), others (6.1%) or unknown (31%). Muscle was removed with stainless steel scissors before the bones were dried to a constant weight.

For Pb analysis, 0.2–0.3 g of the diaphysis without bone marrow was weighed and digested with 2.5 ml of 70% nitric acid at room temperature for 12 h. Two and

a half milliliters of 30% hydrogen peroxide were then added and the temperature was gradually increased up to $160\text{ }^{\circ}\text{C}$ in 1 h and then held at that temperature for 4 h. The digested samples were then diluted to a final volume of 10 ml with deionized water and analyzed by graphite furnace-atomic absorption spectrometry on a Perkin-Elmer model 3300. All of the humerus samples were analyzed, and in those birds in which only a femur was collected, this was analyzed instead. To know whether femur and humerus concentrations were comparable, the humerus and femurs of 30 common buzzards were analyzed. Bone meal (NIST 1486) with a mean Pb concentration of $1.335\pm 0.014\text{ }\mu\text{g/g}$ was analyzed with each batch of 40 samples. The mean (\pm S.E.) Pb concentration determined in this reference material ($n=7$) was $1.381\pm 0.052\text{ }\mu\text{g/g}$. Pb recoveries obtained with spiked blanks ($n=7$) and samples ($n=5$) were 102.7 and 91.7%, respectively. The limit of detection for Pb was approximately $0.01\text{ }\mu\text{g/g}$ of bone. Arsenic was analyzed using a Perkin-Elmer Hydride Generation AAS system model 300, after pre-reduction of an aliquot of the sample with a solution of 10% potassium iodide, 10% hydrochloric acid, and 5% ascorbic acid. The As concentration determined in the reference material ($n=7$) with an indicative value of 6 ng/g was below the detection limit of 22 ng/g. All element concentrations were expressed on a dry-weight basis.

Pb bone concentrations were not normally distributed (Kolmogorov–Smirnov, $P<0.001$), and were log-transformed to fit a normal distribution ($P=0.945$). Pb concentrations in the humerus and femur of the same birds were compared with a paired t -test and the Pearson's correlation coefficient calculated. Pb bone concentrations were compared among species by means of analysis of variance (ANOVA), and post-hoc comparison was done with the Tukey test. Gender and age were also explored in the previous analyses as factors of the ANOVA. Undetected values of As were considered as half of the limit of detection. As concentrations were not log-normally distributed, so the comparison of median concentrations among species was done with a Kruskal–Wallis test. Differences by sex or age were studied with Mann–Whitney U tests in the three species with largest sample size. The relationship between Pb and As concentrations was studied by means of the Pearson's correlation coefficient. Percentages of birds with elevated Pb levels ($>10\text{--}20\text{ }\mu\text{g/g}$), or compatible with lethal Pb poisoning ($>20\text{ }\mu\text{g/g}$) were calculated for each species. These thresholds have been established for waterfowl according to field and experimental data (Pain, 1996).

3. Results

Lead concentrations in the humerus (geometric mean [95% CI]: $0.61 [0.40\text{--}0.92]\text{ }\mu\text{g/g}$) and femur ($0.65 [0.42\text{--}$

1.00] µg/g) of Eurasian buzzard were not significantly different (paired *t*-test, $t_{29}=0.89$, $P=0.379$), and were highly correlated ($r=0.956$, $P<0.001$; Fig. 1). Hence, we considered humerus and femur as equivalent for further analyses. Distribution of bone Pb concentrations was log-normal, ranging from 0.01 to 185.23 µg/g (Fig. 2).

Bone Pb concentrations differed among species (ANOVA, $F_{10, 218}=13.2$, $P<0.001$). The highest concentrations were observed in two scavenger species, red kite and Eurasian griffon. Eurasian buzzard and booted eagle (*Hieraaetus pennatus*) had the lowest Pb concentrations (Table 1). As the effects of sex and age in the model were non-significant and both values were known together in only 39% of the cases, the final interspecific comparison did not include these factors.

Arsenic concentrations were not correlated with Pb, and no differences were observed for sex or age. Simi-

larly to Pb, the highest median As concentration was observed in the red kite (Kruskal–Wallis, $\chi^2_{10}=19$, $P=0.04$; Table 1).

Ten birds of six species had bone Pb concentrations > 10 µg/g, and two birds, one red kite and one Eurasian eagle-owl, had bone Pb concentrations > 20 µg/g (Table 2). The red kite was found in Brunete (Madrid) and admitted to the rehabilitation center of GREFA (Group for the Rehabilitation of Fauna, Madrid) showing dehydration (8–9%), diarrhoea, depression and leg paralysis. Necropsy findings were atrophic myocardium, pale kidneys and urate accumulation in the cloaca. The Eurasian eagle-owl was found in Almuradial (Ciudad Real), but no clinical data was reported by the rehabilitation center apart of being found with

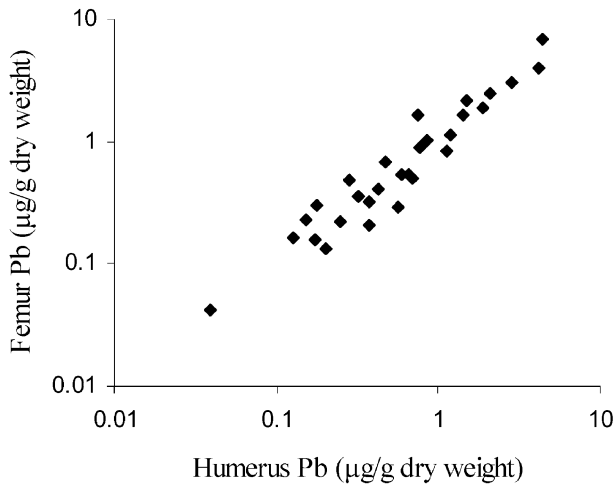


Fig. 1. Relationship between humerus and femur lead concentrations in Eurasian buzzard (*Buteo buteo*) ($n=30$).

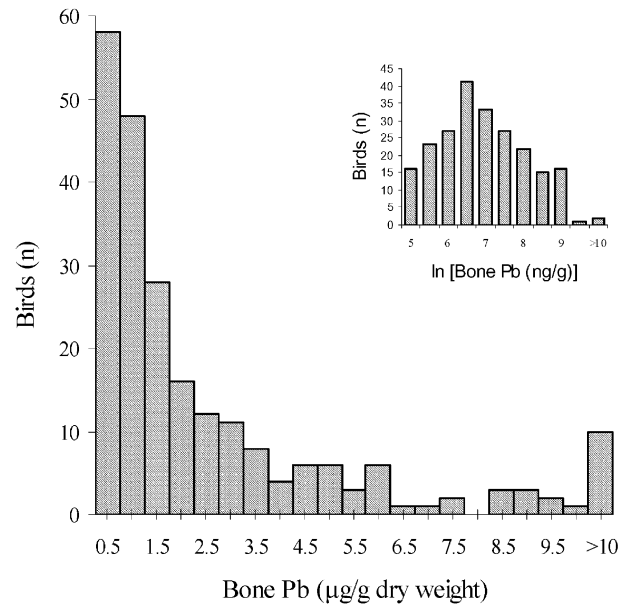


Fig. 2. Distribution of lead bone concentrations in raptors from Spain.

Table 1
Lead (µg/g) and arsenic (ng/g) in bones (dry weight) of birds of prey from Spain

Species	<i>n</i>	Pb			As	
		Geometric mean ^a	95% CI	Range	Median (<i>n</i>) ^b	Range
Red kite <i>Milvus milvus</i>	12	6.00* ^{§†}	3.13–11.51	1.44–38.34	41 (9)	n.d.–1,300
Black kite <i>Milvus migrans</i>	16	1.82*	0.97–3.42	0.20–15.56	n.d. [‡] (6)	n.d.–60
Eurasian griffon <i>Gyps fulvus</i>	4	5.54*	1.97–15.55	2.59–10.31	18 (2)	n.d.–27
Northern goshawk <i>Accipiter gentilis</i>	18	1.57*	1.09–2.27	0.38–5.55	25 (10)	n.d.–51
Eurasian buzzard <i>Buteo buteo</i>	107	0.58	0.47–0.71	0.01–10.25	n.d. [‡] (43)	n.d.–910
Spanish imperial eagle <i>Aquila adalberti</i>	2	2.57	–	0.94–7.00	18 (1)	n.d.–24
Golden eagle <i>Aquila chrysaetos</i>	2	1.56	–	0.49–4.97	n.d. (1)	n.d.–22
Bonelli's eagle <i>Hieraaetus fasciatus</i>	6	1.92	0.74–4.98	0.85–8.73	n.d. [‡] (1)	n.d.–26
Booted eagle <i>Hieraaetus pennatus</i>	11	0.89	0.57–1.39	0.20–1.93	n.d. (5)	n.d.–54
Peregrine falcon <i>Falco peregrinus</i>	9	2.66*	1.31–5.37	0.68–11.50	n.d. [‡] (2)	n.d.–25
Eurasian eagle-owl <i>Bubo bubo</i>	42	2.80* [§]	1.98–3.96	0.33–185.23	17* [‡] (21)	n.d.–170

^a Significantly higher than Eurasian buzzard*, booted eagle[§], and Northern goshawk[†] (Tukey test, $P<0.05$).

^b Medians with occurrence (*n* with value above limit of detection). Significantly lower than red kite[‡] (Kruskal–Wallis test, $P<0.05$).

Table 2
Number of birds (%) exceeding threshold values of bone lead on a dry-weight basis

Species	<i>n</i>	> 10–20 µg/g	> 20 µg/g
Red kite	12	4 (33.3)	1 (8.3)
Black kite	16	1 (6.3)	–
Eurasian griffon	4	1 (25)	–
Eurasian buzzard	107	1 (0.9)	–
Peregrine falcon	9	1 (11.1)	–
Eurasian eagle-owl	42	–	1 (2.4)

traumatism close to a road. In the birds with > 10 µg/g bone Pb, clinical data noted the presence of visceral gout in one Eurasian griffon and strychnine and carbonyl poisoning in two of the red kites.

4. Discussion

The use of bone analysis for identifying elevated Pb exposure in raptors has been frequently discussed. Wayland et al. (1999) found that the bone Pb concentrations in wild, bald and golden eagles were related to those in the liver, and although these authors considered bone Pb as a poor predictor of liver Pb ($r=0.47$), birds with liver Pb concentration > 6 µg/g had higher bone Pb concentration than those with liver Pb < 6 µg/g (9.7 vs. 4.1 µg/g). Custer et al. (1984) obtained a high correlation between liver and bone Pb concentrations ($r=0.9$) in American kestrels, and Wickson et al. (1992), Pain et al. (1992) and Mateo et al. (2001b) have all reported significant correlations in several waterfowl species ($r=0.47–0.82$). Bone Pb concentrations can be considered as a good predictor of Pb shot ingestion interspecifically (Pain et al., 1992) or at population level (Stendell et al., 1979; Scheuhammer and Dickson, 1996).

Experimental data suggests that bone Pb concentrations may also be helpful for identifying abnormal Pb exposure at an individual level as established for waterfowl by Pain (1996). Bald eagles experimentally Pb-shot poisoned accumulated a mean of 11 µg/g of Pb in the femur, tibia and humerus, while a control bird had 6 µg/g (Pattee et al., 1981). Raptors of different species dosed with 3 mg of Pb/kg body weight/day for 30 weeks accumulated 40.1 µg/g of Pb in humerus, while control birds had 28.4 µg/g (Reiser and Temple, 1981). Although the Pb bone concentration in these controls was above the background level in waterfowl (10 µg/g), their blood Pb level was also above the background established for birds (0.2 µg/ml; Franson, 1996; Pain, 1996), and they could have been exposed to Pb in the wild before the beginning of the experiment. American kestrels bred in captivity and exposed experimentally to 0.5, 120, 212 and 448 µg/g of dietary Pb for 60 days

accumulated 1.67, 7.84, 16.6 and 18.44 µg/g of Pb in femur, respectively (Custer et al., 1984). In that study, background levels in liver and bone established for waterfowl (6 and 10 µg/g, respectively; Pain, 1996) were both exceeded in the kestrels exposed to 212 µg/g, and toxic liver Pb concentration established for raptors (9 µg/g; Franson, 1996) was exceeded at the highest dose. However, no effects were observed on body weight, hematocrit, hemoglobin or erythrocyte counts, suggesting important differences in susceptibility to Pb poisoning among species (Custer et al., 1984). This resistance to Pb poisoning was also observed in American kestrels fed with mice containing Pb shot for 60 days, that accumulated 28.7 µg/g of Pb in tibia (4.2 µg/g for controls), but only 0.37 µg/g in liver (Stendell, 1980). In another study, American kestrels exposed to 50 µg/g of Pb in their diet for 5–7 months accumulated 13.5 µg/g of Pb in humerus (3.5 µg/g for controls), and showed a depression of delta-aminolevulinic acid dehydratase activity of 80% with liver Pb concentration below the threshold of abnormal exposure (Franson et al., 1983; Patte, 1984). According to the reviewed data, bone Pb concentration > 10 µg/g can be a good indicator of an abnormal exposure to Pb during the life of an individual, but the use of bone Pb concentration to diagnose Pb poisoning should be accompanied by clinical signs or necropsy findings.

Bone Pb concentrations in the present study reflect the differences among species in Pb exposure that may be explained by feeding habits and potential Pb shot ingestion. The highest bone Pb concentrations were found in two species that usually feed on carrion, such as red kite and Eurasian griffon (Table 3). One Eurasian griffon with 10.3 µg/g of Pb in bone showed visceral gout, a clinical sign associated with Pb poisoning in raptors (Locke and Thomas, 1996). Pb poisoning has been described in one Eurasian griffon in Spain (Mateo et al., 1997), as well as other species of vultures elsewhere (Wiemeyer et al., 1988). Six Eurasian griffons admitted to wildlife rehabilitation centers in Spain had blood Pb levels above the background threshold of 0.2 µg/ml (García-Fernández et al., 1995). Eurasian griffon in Spain feeds mainly on the carrion of domestic and wild ungulates. The latter may contain bullet fragments or shot pellets in their flesh. No cases of Pb poisoning in red kite in Spain had been reported before this study, but bone Pb concentration of 38 µg/g together with the clinical observations reported by the wildlife rehabilitation center suggest that Pb poisoning was the cause of death for one of the red kites studied herein. A high proportion (42%) of red kite bones had Pb concentrations above the background level (> 10 µg/g). As this species is known to be susceptible to Pb poisoning because of its scavenging habits, pellet surveys have previously been conducted. García and Viñuela (1999) found Pb shot in 1% of red kite pellets from upland

Table 3
Summarized information on the studied raptor species in Spain

Species	Breeding pairs ^a	Population trend ^{a,b}	Cases of Pb poisoning	Diet ^c
Red kite	3338–4054	–1	+ ^d	C,s–mM,s–mB
Black kite	9000	0		C,s–mM,s–mB, F
Eurasian griffon	8074	+2	+ ^{d,e}	C
Northern goshawk	2300–3000	–1		S–mB, s–mM
Eurasian buzzard	5500	–1	+ ^f	S–mM, R, C
Spanish imperial eagle	131	+2	+ ^g	mM, mB, R, C
Golden eagle	1192–1265	–1	+ ^h	mM, mB, R, C
Bonelli's eagle	627–695	–1		mM, mB
Booted eagle	2000–4000	0		S–mB, s–mM
Peregrine falcon	1628–1751	–1		S–mB
Eurasian eagle-owl	520–600	–1	+ ^d	S–mM, s–mB

^a BirdLife International/European Bird Census Council (2000).

^b Population trend for the period 1970–1990. +2=large increase of at least 50%; +1=small increase of 20–49%; 0=stable, with overall changes less than 20%;-1=small decrease of 20–49%.

^c Data on diet from del Hoyo et al. (1994, 1999). s=small; m=medium; B=birds; M=mammals; C=carrion; F=fish; R=reptiles.

^d Present study.

^e Mateo et al. (1997).

^f Guitart et al. (1999).

^g Hernández (1995).

^h Cerradelo et al. (1992).

areas in the center of Spain, and the percentage at some locations during the autumn (7%) actually surpassed the level observed in the Guadalquivir marshes (5.5%), where kites mostly feed on geese carcasses (Mateo et al., 2001a). The black kite (*M. migrans*) has similar feeding habits to the red kite, but its presence in Spain does not overlap with the hunting season and consequently it may be less exposed to shot prey than are the resident or wintering red kites. Pb shot ingestion has been more frequently observed in different raptor species during the hunting season (autumn–winter) (Pain et al., 1997; Mateo et al., 1999, 2001a). Eurasian marsh-harrier is another opportunistic and scavenger species present in Spain with 52.5% of birds from the Ebro delta having blood Pb levels >200 ng/ml, and 4 out of 7 birds containing bone Pb levels >10 µg/g (Mateo et al., 1999). One marsh harrier found dead in France containing ingested Pb shot, had Pb concentrations of 54.9 µg/g in the liver and 15.6 µg/g in the bone, and three birds out of 10 had bone Pb levels >10 µg/g (Pain et al., 1993). Similarly, other scavengers such as the Eurasian *Haliaeetus* species that feed on deer offal and waterfowl carcasses are reported elsewhere as susceptible to Pb poisoning (Kim et al., 1999; Kenntner et al., 2001).

Although little clinical information was available on the Eurasian eagle-owl with 185 µg/g of Pb in its bone, this extremely high concentration again suggests the bird suffered from Pb poisoning that could have contributed to the death by a collision with a car. This is also the first report of potential Pb poisoning in the Eurasian eagle-owl, a species that feeds on live mammals (mostly rabbits) and birds of a size similar to those usually shot by hunters (Table 3). García-Fer-

nández et al. (1997) reported higher bone Pb levels in nine Eurasian eagle-owls (15.4 µg/g) than in six Eurasian buzzards (2 µg/g) from southeastern Spain. Eurasian eagle-owl may prey frequently on animals with physical impairments (Fernández-Llario and Hidalgo de Trucios, 1995) and further dietary studies of this species should pay attention to the presence of Pb shot in the regurgitated pellets. Other large raptors, such as golden eagle, Spanish imperial eagle and Bonelli's eagle (*Hieraaetus fasciatus*), that also feed on rabbits, have been included in the present study but with only a small sample size. Bone Pb concentrations were not high, although Pb poisoning has previously been described in the first two of these species in Spain, and in the case of the golden eagle was due to the ingestion of a rabbit with embedded shot pellets (Cerradelo et al., 1992; Hernández, 1995).

Peregrine falcon (*F. peregrinus*) may be another species susceptible to Pb poisoning because it consumes live birds (pigeons or partridges), which may have embedded Pb shot in their flesh. There is no data available on the occurrence of embedded Pb shot in upland birds, but this value ranges from 10 to 68% in waterfowl species (Scheuhammer and Norris, 1996). Pain et al. (1995) detected liver Pb concentrations >6 µg/g in 4 out of 26 peregrine falcons, and one bird with a liver Pb level of 22 µg/g which was likely to have died from Pb poisoning. Northern goshawks (*Accipiter gentilis*) had bone Pb concentrations below 10 µg/g in this study, but one was found in France with a liver Pb concentration of 711 µg/g (Pain and Amiard-Triquet, 1993).

The lowest Pb exposure levels were detected in raptors that usually feed on small prey, such as the Eurasian

buzzard and the booted eagle (Table 3). Only one of 107 Eurasian buzzards had $>10 \mu\text{g/g}$ of Pb in its bone, and it was a young bird illegally removed from the nest that could have been fed in captivity with shot prey before its admittance to the rehabilitation center. General awareness of Pb shot toxicity is scarce and hence several cases of Pb poisoning in falconry birds are reported in the literature (MacDonald et al., 1983). Cases of Pb poisoning in Eurasian buzzard have been diagnosed in both Britain and Spain (Pain et al., 1995; Guitart et al., 1999), and liver Pb concentrations $>6 \mu\text{g/g}$ were found in five of 90 buzzards from France (Pain and Amiard-Triquet, 1993). Eurasian buzzards feed in Spain on

invertebrates, reptiles, birds, small mammals and young rabbits (Mañosa and Cordero, 1992), but also occasionally on carrion (Table 3) and consequently may be at some risk of Pb shot ingestion.

Arsenic levels were highest in one of the scavenger species as was observed for Pb. Arsenic is present in small amounts in Pb shot (Hall and Fisher, 1985a), and although Pb shot ingestion does not tend to affect liver As concentrations in birds (Hall and Fisher, 1985b; Pain et al., 1992), bone and feather As levels may be increased (Hall and Fisher, 1985a). Hall and Fisher (1985a) failed to find higher As concentrations in bones and feathers of waterfowl with ingested shot, but

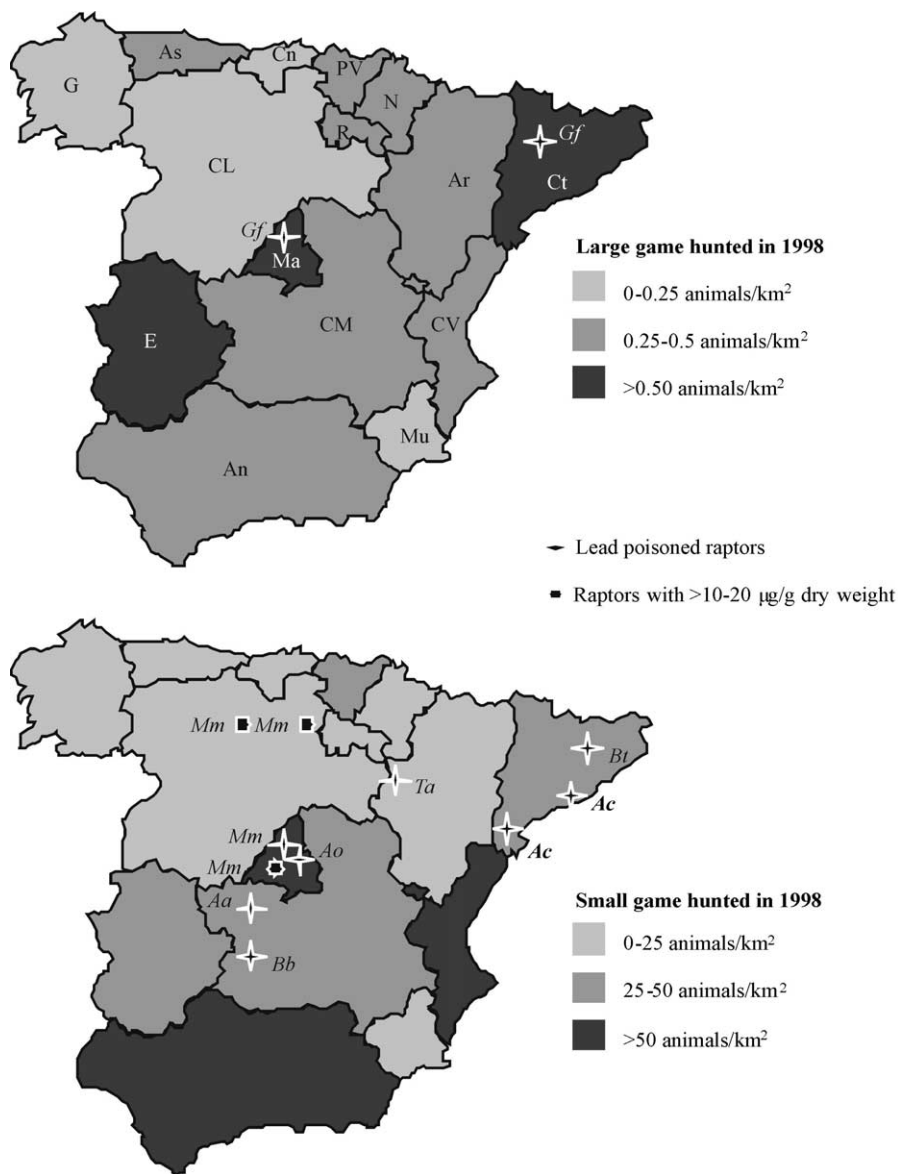


Fig. 3. Hunting pressure in continental Spain by autonomous communities (G: Galicia, As: Asturias, Cn: Cantabria, PV: País Vasco, N: Navarra, Ar: Aragón, Ct: Cataluña, CL: Castilla y León, Ma: Madrid, R: La Rioja, CV: Comunidad Valenciana, E: Extremadura, CM: Castilla-La Mancha, An: Andalucía, Mu: Murcia). Reported cases of lead exposure and poisoning marked with symbols (Mm: red kite *Milvus milvus*, Gf: Eurasian griffon *Gyps fulvus*, Bt: Eurasian buzzard *Buteo buteo*, Aa: Spanish imperial eagle *Aquila chrysaetos*, Ta: common barn-owl *Tyto alba*, Bb: Eurasian eagle-owl *Bubo bubo*, Ao: Northern long-eared owl *Asio otus*).

observed a significant correlation between Pb and As in bones of several species ($r=0.68\text{--}0.97$). Arsenate is a structural analog of phosphate that may substitute for it in the apatite crystal of bone (WHO, 2001). Despite evidence that Pb shot is a possible source of As, one Eurasian buzzard with high As concentration had low Pb level, suggesting the presence of other As sources for raptors.

Less is known about the ecological effects of Pb shot in upland areas than in wetlands (Kendall et al., 1996). The use of Pb shot in wetlands is minimal (30–50 t) compared to annual use of Pb shot in Spain (5000 t) (Guitart et al., 1999). Hunting pressure by autonomous communities in continental Spain is shown in Fig. 3, and Madrid, where several cases of Pb poisoning have been described, has one of the highest values. In 1998, 177,339 large mammals, 6,436,862 medium-small mammals, and 14,262,260 birds were hunted in Spain (MAPA, 2001). Clinical cases of Pb poisoning have been described in 8 species of raptors in Spain (Fig. 3), but Pb shot was only banned recently (in 2001) for waterfowl hunting in protected areas. It is difficult to assess the relevance of the mortality due to Pb poisoning in raptors, and our data suggests it is well behind electrocution, shooting, traumatism or illegal poisoning. However, Pb poisoning in our sample could be underestimated. The probability of retrieving birds dead by the cited causes may be higher, because their effect is immediate and birds tend to concentrate near power lines, roads or open fields, while development of Pb poisoning takes several days and birds may become more unobtrusive until death. Further research should be conducted to look at the availability of prey with embedded shot for raptors in upland habitats. A monitoring program should be set up to observe Pb levels in species known to be vulnerable to Pb poisoning, especially those species with small populations or declining trends (Table 3), such as the Spanish imperial eagle, golden eagle, red kite and Eurasian eagle-owl.

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