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Levels of heavy metals and metalloids in critically endangered Iberian lynx and other wild carnivores from Southern Spain

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ABSTRACT

The Iberian lynx (*Lynx pardinus*) is the most endangered felid in the world with a wild population which probably stands at less than 200 individuals inhabiting two areas in Southern Spain (Doñana and Sierra Morena) that are known to have been contaminated by heavy metals and metalloids due to a long history of mining activities. This contamination may pose a threat to long term conservation efforts and hence, the concentrations of seven elements (As, Se, Cd, Zn, Cu, Pb, Hg) were determined in the liver, muscle and bone of 9 lynx, as well as 17 red foxes (*Vulpes vulpes*), 11 Egyptian mongooses (*Herpestes ichneumon*), 4 common genets (*Genetta genetta*) and 1 Eurasian badger (*Meles meles*). The mean concentrations found were below the threshold levels indicative of chronic intoxication in all the species studied. In general, genet and red fox were species with the highest concentrations of several elements in Doñana, whilst Iberian lynx had the lowest levels of most of them. Lynx from Sierra Morena had significantly higher concentrations of bone Pb (2.05 μg/g d.w.) than those from Doñana (0.13 μg/g d.w.), probably due to the mineralised underlying geology and/or the abandoned mine workings in Sierra Morena. Egyptian mongoose presented liver concentrations of Hg up to 9.7 μg/g d.w. A strong relationship between Hg and Se levels was found in liver and muscle samples of all the studied species, especially in mongoose. In conclusion, levels of the studied elements do not appear to represent a significant threat for the lynx or for the other carnivores studied. However, given the critical status of the Iberian lynx, a continuous monitoring scheme remains necessary.
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1. Introduction

The Iberian lynx (*Lynx pardinus*) is, according to the World Conservation Union (IUCN), the most endangered felid species in the world. Recent studies have estimated that only 160–200 lynx may inhabit two areas in Southern Spain, that is, Sierra Morena and Doñana (Guzmán et al., 2004). The Iberian lynx apparently experienced one or several Pleistocene demographic bottlenecks which acted to reduce levels of mitochondrial sequence variation and levels of microsatellite size variation in comparison to most other felid species (Johnson et al., 2004). Further, inbreeding was recently proposed as a cause of the loss of effectiveness of the immune system observed in lynx (Peña et al., 2006). Thus, remnant populations of this species might be particularly vulnerable to the effect of environmental stressors such as chemical contaminants.

A study of heavy metals in mammals of Doñana carried out in 1982–83, including five Iberian lynx, reported that none of

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the metals investigated was found in quantities high enough to promote disease, although Hg levels of 1.3 μg/g were found in the liver of European otters (*Lutra lutra*) (Hernández et al., 1985). However, a mining spill in Aznalcóllar in 1998 released more than 6,000,000 m^3 of acidic waste containing a broad spectrum of metals such as Pb, Zn, Fe, As, Cu, Sb, Co, Tl, Bi, Cd, Ag, Hg, and Se into the Agrio-Guadiamar river system. The toxic spill flooded more than 4000 ha of riverbank and adjacent land, and had an impact on the Doñana Natural and National Park areas (Grimalt et al., 1999; Vanderlinden et al., 2006). The adverse effects of the Aznalcóllar spill have been studied in several species of terrestrial vertebrates in Doñana, such as Algerian mouse (*Mus spretus*) (Tanzarella et al., 2001; Bonilla-Valverde et al., 2004), white stork (*Ciconia ciconia*) and black kite (*Milvus migrans*) (Pastor et al., 2004; Smits et al., 2005; Baos et al., 2006), and greylag goose (*Anser anser*) (Mateo et al., 2006). However, no post spill data are available regarding the concentration of heavy metals in carnivorous mammals, which are at the top of the food web and may therefore be very sensitive to any biomagnification processes that may be occurring. In addition to pollution related to the Aznalcollar spill, Doñana lies in close proximity to the Ría (estuary) of Huelva, which is one of the most highly industrialised areas in Spain, where several metallurgic, petrochemical and fertilizer industries are located. Relatively high levels of P, As, Cu, Pb and Ti have been determined in the air of a monitoring station bordering the Doñana National Park in comparison with other rural sites in Spain (Sánchez de la Campa et al., 2007).

In the second key area where the lynx is still present, Sierra Morena, there is also the risk of heavy metal exposure for carnivores, since numerous mines, mainly of Pb and Zn, were exploited in this region up until 1988, many of which now lie abandoned having undergone little or no post operational remediation (Palero-Fernández and Martín-Izard, 2005). Moreover, the Almadén district, close to Sierra Morena, has produced one third of the total world production of Hg in its lifetime, and is one of the world's largest Hg-contaminated sites which continues to generate significant atmospheric emissions (Higueras et al., 2006).

Some heavy metals and metalloids such as Pb, Cd, Hg and As are highly toxic and may cause lethal poisoning. Although other elements like Zn, Cu or Se are essential for the health and growth of animals, they may also be toxic at high exposure doses. The toxic effects of heavy metals and metalloids are broad ranging, and include, among others, carcinogenicity, impaired reproduction and teratogenicity, immunosuppression, cardiovascular and pulmonary diseases, nephrotoxicity and neurotoxicity (Wren, 1986; Goyer, 1996). For example, methylated forms of Hg which accumulate in aquatic ecosystems have been shown to have a toxicological impact on the American mink (*Mustela vison*) (Osowski et al., 1995) or the endangered Florida panther (*Felis concolor coryi*) (Newman et al., 2004). Roelke et al. (1991) found a significant inverse correlation between the amount of mercury in the whole blood of lactating females of Florida panther, and the survival of offspring up to 6 months of age. Thus, Hg and other heavy metals may be a threat for other felids inhabiting wetland areas, such as the endangered Iberian lynx.

The present study aims to provide new data on the concentrations of selected environmental contaminants (As, Se, Cd, Zn, Cu, Pb, Hg) in lynx and sympatric wild carnivores to determine if any of these elements pose a threat for the conservation of the Iberian lynx, which now sits precariously at the brink of extinction.

2. Materials and methods

2.1. Study areas

The Sierra Morena area (Northern Andalusia, Southern Spain, 38°13′N, 4°10′W) includes two contiguous protected Natural Parks and several private hunting estates, and comprises an area of 1125 km^2 with elevations between 500 and 1300 m. The climate is Mediterranean subhumid with marked seasons, and Mediterranean shrubland is dominant. The Doñana area (South-western Andalusia, 37°0′N, 6°30′W) comprises about 870 km^2, mostly within the Doñana National and Natural Parks. It is a humid area limited by the Atlantic Ocean to the West, the Guadalquivir River to the East, and crops extending to the North for several kilometres. The area is flat and mostly near sea level, the climate is also Mediterranean subhumid. Three ecosystem types are predominant: fixed dunes, mobile dunes and marshes. Vegetation in the fixed dunes is a mixture of autochthonous Mediterranean shrubland, pines woodland, and has *Eucalyptus camaldulensis* plantations in some areas.

2.2. Sampling

From 2004 to 2006, we necropsied 42 free-living carnivores: 9 Iberian lynx, 17 red foxes (*Vulpes vulpes*), 11 Egyptian mongooses (*Herpestes ichneumon*), 4 common genets (*Genetta genetta*) and 1 Eurasian badger (*Meles meles*). Most of the animals were road-killed, with the exception of 14 foxes that were hunted. The lynx came from Sierra Morena ($n=3$) and Doñana ($n=6$). Individuals of the other species studied, all came from Doñana. Animals were sexed, and the age was estimated by tooth wear and an evaluation of facial, body, and pelt features. Only one lynx was younger than 1 year old (about 6 months); for foxes, we sampled 9 adults and 7 cubs (≤1 month); all mongoose and genets were adult individuals; and the badger was a juvenile. At necropsy, we carefully took samples of liver, bone, and muscle, and stored them at −20° until laboratory analyses.

2.3. Laboratory analysis

Samples were freeze dried (Christ Alpha 1–2, Braun Biotech) and 0.3–0.5 g were digested with 3 ml of HNO_3 (69% Analytical Grade, Panreac), 1 ml of H_2O_2 (30% v/v Suprapur, Merck) and 4 ml of H_2O (Milli-Q grade) with a microwave oven (Ethos E, Milestone). The program for the digestion began at a potency of 750 W then ramped for 15 min up to 180 °C, after which, samples were held for 10 min at 800 W at a temperature of 180 °C. Digested samples were diluted to a final volume of 50 ml with Milli-Q H_2O. The analyses of As, Se, Cd and Pb were done by graphite furnace-atomic absorption spectroscopy using an AAnalyst 800 (Perkin Elmer) equipped with an autosampler AS 800 (Perkin Elmer) and utilising 50 μg $NH_4H_2PO_4$ and 3 μg $Mg(NO_3)_2$ as matrix modifiers in each atomization for

Pb and Cd and 5 μg Pd and 3 μg $Mg(NO_3)_2$ for As and Se. The analyses of Cu and Zn were done by flame atomization in an AAnalyst 800 equipped with an autosampler AS 90plus (Perkin Elmer). Mercury was analyzed by the cold vapour technique with a flow injection system coupled to an atomic absorption spectrophotometer FIMS-400 (Perkin Elmer) equipped with an autosampler AS 900 (Perkin Elmer). Calibrations were prepared from commercial solutions with 1 g/l of each element (Panreac). The limits of detection (LODs, in μg/g dry weight, back-calculated to in tissue concentrations) were, for As: 0.036 in liver and 0.222 in muscle and bone, Se: 0.1, Cd: 0.006, Zn: 5.56, Cu: 4.66, Pb: 0.073 and Hg: 0.01. Blanks were processed in each batch of digestions.

A reference sample of bovine liver (BCR 185R, Community Bureau of Reference) was analyzed (n=8) and the recovery (mean % recovery±S.E.) was 94.4±5.8% for Pb, 95.8±0.7% for Zn, 100.4±0.6% for Cu, 99.0±4.0% for Cd, and 74.3±2.2% for Se (the certified level of As in this standard was below the LOD, and no Hg certified value is available). A reference sample of bone ash (SRM 1400, National Institute of Standards and Technology, USA) with certified levels of Pb and Zn was analyzed (n=12) and the recovery was 94.5±1.8% for Pb and 92.6±1.3% for Zn. A reference sample of pig kidney (BCR 186, Community Bureau of Reference, European Commission) with certified levels of Hg and Se was analyzed (n=2) and the recovery was 99.5±1.0% and 86.5±7.8%, respectively. A reference sample of dogfish muscle (DORM-2, National Research Council, Canada) was also analyzed for Hg (n=3) and the recovery was 94.5±1.6%. All concentrations are given in dry weight (d.w.), but can be converted to wet weight for comparative purposes (with the results of other studies) given that the average (±SD) dry weights were 32.5±4.5% in liver, 29.3±4.1% in muscle and 85.7±9.4% in bone.

2.4. *Statistical analysis*

Data below the detection limits were assigned values of half of the respective LOD for each element. The concentrations of the elements were log-transformed to approach a normal distribution of the data. Differences among species and the effects of sex and age of the animals from Doñana only, were studied with a general linear model (GLM) with these three factors as independent variables. As lynx were sampled in two locations, Mann–Whitney U tests were used to compare the concentrations of elements between Doñana and Sierra Morena. This non-parametric test was also used to compare concentrations between juveniles and adults of red fox, and between sexes of the species with largest sample size (i.e., red fox and mongoose). The relationships between the log-transformed concentrations of each element in different tissues or between different elements in each tissue were studied by partial correlations controlling for species with all the data, and separately with the species with largest sample size. Statistical significance was set at $p<0.05$.

3. Results

The studied elements were detected in all the tissues, except for Cd in bone, where all data were below the LOD (Tables 1–3). Differences in concentrations between species from Doñana were observed for Se and Pb in liver ($F_{3,\ 26}=6.17$, $p=0.003$; $F_{3,\ 26}=6.41$, $p=0.002$; respectively); in bone for Zn ($F_{3,\ 26}=7.07$, $p=0.001$) and Cu ($F_{3,\ 26}=4.65$, $p=0.01$); and in muscle for Se ($F_{3,\ 28}=47.61$, $p<0.001$), Cu ($F_{3,\ 28}=4.83$, $p=0.008$) and Hg ($F_{3,\ 28}=6.73$, $p=0.001$). In general, genet and red fox were the species with highest concentrations of several elements, whilst Iberian lynx had the lowest levels of most of them. Bone Pb concentrations were higher in lynx from Sierra Morena than in Doñana ($Z=2.12$, $p=0.034$; Table 2).

Females had higher concentrations in liver of Se ($F_{1,\ 26}=18.32$, $p<0.001$), Cd ($F_{1,\ 26}=6.04$, $p=0.021$), Pb ($F_{1,\ 26}=7.53$, $p=0.011$), and Hg ($F_{1,\ 26}=8.59$, $p=0.007$) than males in the statistical analyses including all the studied species. This difference by sex for the species with largest sample size

Table 1 – Concentrations (geometric mean and range in μg/g dry weight) of heavy metals and metalloids in liver of wild carnivores from Southern Spain

	Iberian lynx		Red fox	Egyptian mongoose	Common genet	Eurasian badger
	Doñana	S. Morena	Doñana	Doñana	Doñana	Doñana
	(n=3)	(n=2)	(n=16)	(n=11)	(n=4)	(n=1)
As	nd	0.041	0.047	0.019	0.137	nd
		nd–0.093	nd–0.449	nd–0.045	nd–0.321	
Se	1.54^B	1.52	1.72^B	2.02^B	5.32^A	4.88
	0.96–2.16	1.29–1.79	0.91–14.94	1.16–3.62	1.18–10.41	
Cd	0.122	0.057	0.113	0.131	0.040	0.017
	0.050–0.254	0.037–0.088	nd–1.425	0.029–0.521	0.011–0.141	
Zn	114.7	152.8	136.7	128.1	122.9	144.3
	97.2–30.4	108.6–215.0	75.7–307.0	91.5–322.5	107.3–143.3	
Cu	18.2	35.4	72.0	16.9	19.4	89.5
	13.3–28.1	22.5–55.8	6.9–951.5	9.6–33.6	10.5–58.7	
Pb	0.043^B	0.248	0.257^A	0.054^B	0.046^B	0.460
	nd–0.057	0.088–0.698	nd–0.908	nd–0.388	nd–0.087	
Hg	0.233	0.135	0.393	1.753	0.652	0.090
	0.100–0.618	0.123–0.149	nd–5.363	0.242–9.686	0.155–2.228	

[A, B]Mean values sharing a letter in superscript did not differ significantly among species from Doñana (LSD test). Where no superscript letters are presented, no differences were detected. No differences were found between Iberian lynx from Doñana and Sierra Morena.

Table 2 – Concentrations (geometric mean and range in µg/g dry weight) of heavy metals and metalloids in bones of wild carnivores from Southern Spain

	Iberian lynx		Red fox	Egyptian mongoose	Common genet	Eurasian badger
	Doñana	S. Morena	Doñana	Doñana	Doñana	Doñana
	($n=4$)	($n=2$)	($n=16$)	($n=11$)[1]	($n=4$)[2]	($n=1$)
As	0.368	0.333	0.510	0.467	0.593	0.348
	0.313–0.409	0.111–0.619	nd–1.030	nd–1.063	0.406–0.880	
Se	0.357	0.306	0.786	0.431	0.810	1.077
	nd–1.406	0.057–1.082	0.134–1.472	nd–1.361	0.214–1.375	
Cd	nd	nd	nd	nd	nd	nd
Zn	133.1^{B}	130.7	142.1^{B}	145.0^{B}	173.1^{A}	128.3
	120.5–155.7	123.0–137.2	111.7–163.1	131.6–173.7	152.8–198.4	
Cu	1.18^{B}	0.54	4.20^{A}	0.63^{B}	1.61^{AB}	1.02
	nd–7.85	0.39–1.03	nd–21.15	nd–7.67	nd–8.48	
Pb	0.136^{a}	2.052	0.385	0.136	0.112	0.735
	nd–0.411	1.446–2.743	nd–2.550	nd–0.358	nd–1.055	
Hg	nd	nd	0.012	0.011	0.023	–
			nd–0.038	nd–0.016	nd–0.052	

[1]$n=6$ for Hg; [2]$n=2$ for Hg; $^{A,\ B}$Mean values sharing a letter in the superscript did not differ significantly among species from Doñana (LSD test). Where no superscript letters are presented, no differences were detected.
^{a} A significant difference between lynx from Doñana and Sierra Morena was detected.

(mongoose and fox; Fig. 1) was only significant for Pb in mongoose ($Z=2.57$, $p=0.01$). No differences were found between sexes in bone and muscle. Adult foxes presented higher levels of Cd in liver ($Z=2.71$, $p=0.007$) and muscle ($Z=2.65$, $p=0.008$), Pb in bone ($Z=2.32$, $p=0.02$), and Hg in muscle ($Z=2.31$, $p=0.021$) (Fig. 2).

The partial correlations between muscle and liver were significant for As ($r=0.534$, $p=0.001$), Se ($r=0.434$, $p=0.009$), Cd ($r=0.463$, $p=0.004$), Zn ($r=0.388$, $p=0.021$), and Hg ($r=0.822$, $p<0.001$). The partial correlations between muscle and bone were significant for Cu ($r=0.647$, $p<0.001$).

Partial correlations between elements were similar for liver and muscle. Figs. 3 and 4 represent those with $r>0.5$. A strong relationship between Hg and Se levels was found in the liver ($r=0.514$, $p=0.001$; Fig. 3) and in the muscle ($r=0.449$, $p=0.003$) of all the studied species. A positive relationship was also found between the concentrations of Hg and Cd in liver ($r=0.732$, $p<0.001$; Fig. 4) and muscle ($r=0.391$, $p=0.011$). Arsenic was correlated with Cu in liver ($r=0.43$, $p=0.009$) and muscle ($r=0.379$, $p=0.014$). Some other correlations were only significant for one of these soft tissues. In liver, the correlation was significant between Pb and Zn ($r=0.431$, $p=0.009$), and Pb

Table 3 – Concentrations (geometric mean and range in µg/g dry weight) of heavy metals and metalloids in muscle of wild carnivores from Southern Spain

	Iberian lynx		Red fox	Egyptian mongoose	Common genet	Eurasian badger
	Doñana	S. Morena	Doñana	Doñana	Doñana	Doñana
	($n=6$)	($n=2$)	($n=17$)	($n=11$)	($n=4$)	($n=1$)
As	0.202	nd	0.416	0.139	0.280	nd
	nd–1.548		nd–4.734	nd–1.336	nd–0.662	
Se	0.298^{C}	0.355	0.535^{B}	0.563^{B}	3.211^{A}	1.832
	nd–0.613	0.255–0.438	0.271–0.798	0.446–0.743	1.425–6.445	
Cd	0.004	nd	0.007	0.007	0.007	0.003
	nd–0.009		nd–0.047	nd–0.039	nd–0.058	
Zn	88.7	125.0	118.7	112.2	128.1	158.2
	55.1–131.1	111.7–143.5	80.7–206.9	72.7–192.8	78.4–228.1	
Cu	1.07^{B}	0.90	8.64^{A}	4.33^{A}	3.71^{AB}	8.15
	nd–9.42	nd–2.16	nd–22.30	nd–12.58	nd–15.51	
Pb	0.075	nd	0.053	0.038	0.049	0.037
	nd–0.392		nd–0.285	nd–0.045	nd–0.112	
Hg	0.024^{C}	0.017	0.161^{B}	0.601^{A}	1.543^{A}	0.069
	nd–0.146	nd–0.022	nd–1.769	nd–2.634	0.281–3.831	

$^{A,\ B,\ C}$Mean values sharing a letter in the superscript did not differ significantly among species from Doñana (LSD test). Where no superscript letters are presented, no differences were detected. No differences were found between Iberian lynx from Doñana and Sierra Morena.

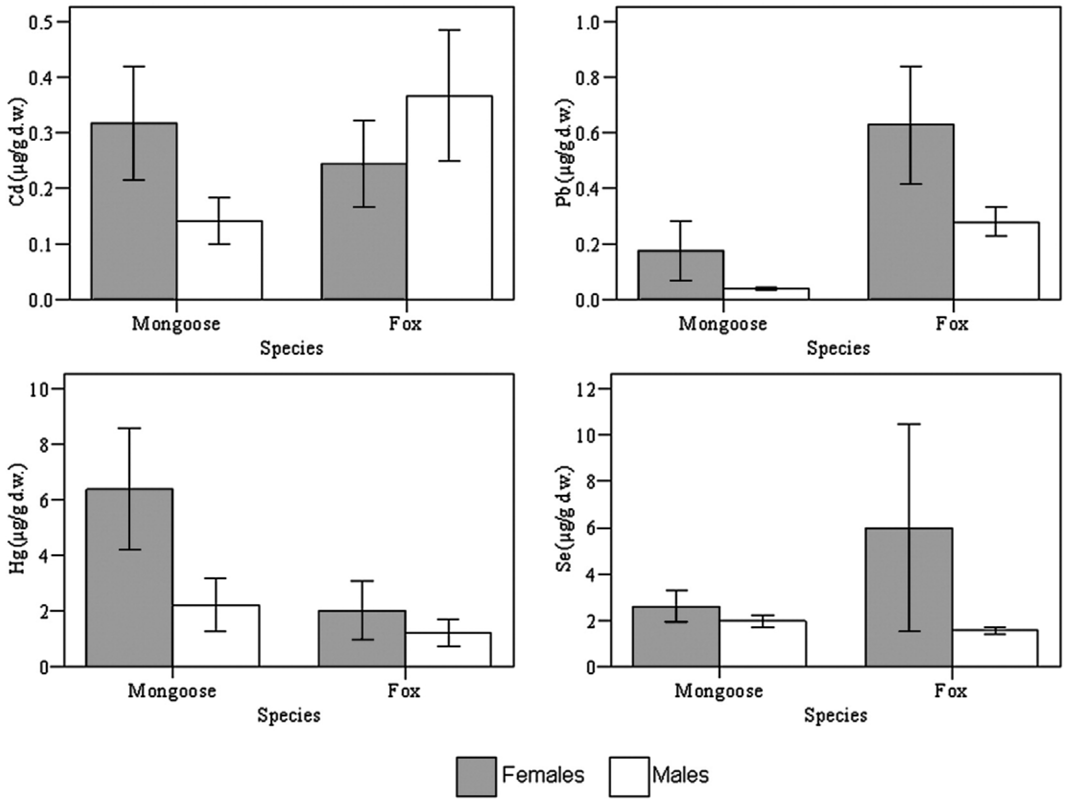

Fig. 1 – Differences by sex in liver concentrations (mean ± S.E.) of Cd, Pb, Se and Hg. For Egyptian mongoose, n=3 females and 8 males. For red fox, n=3 females and 13 males.

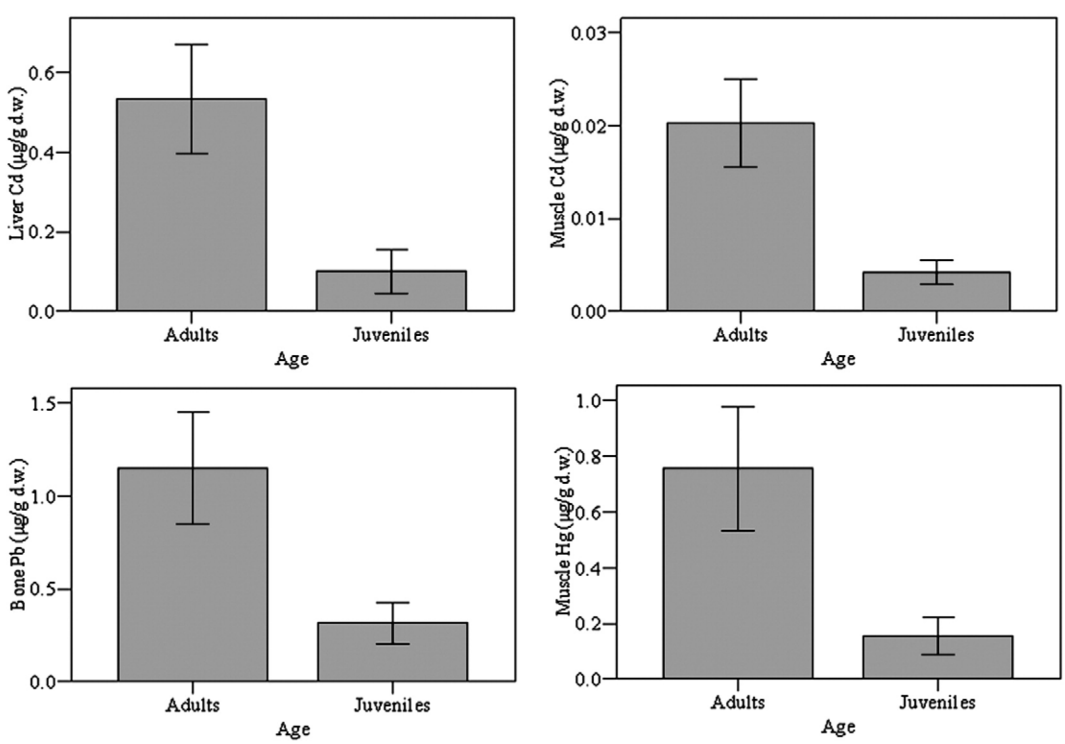

Fig. 2 – Differences by age in the concentrations (mean ± S.E.) of Cd, Pb and Hg in tissues of red fox, n=9 adults and 8 juveniles, except for liver (where n=9 and 7) and bone (where n=8 and 8).

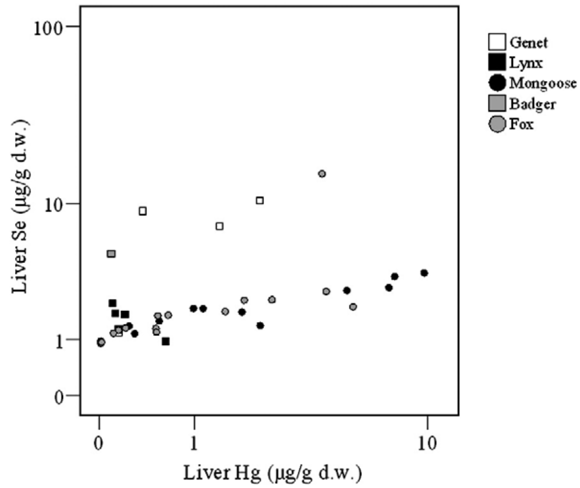

Fig. 3 – Correlation between Hg and Se concentrations in livers of different species of carnivores. The strongest regressions were observed with the data for the red fox (r=0.702, p=0.002) and the Egyptian mongoose (r=0.899, p<0.001).

and Cu (r=0.406, p=0.014). In muscle, the correlation was significant between Pb and As (r=0.361, p=0.02), As and Hg (r=0.324, p=0.039), Se and Cu (r=0.347, p=0.026), Cu and Hg (r=0.485, p=0.001), and Cd and Cu (r=0.43, p=0.005).

4. Discussion

As far as we know, this is the first study that has tested the concentrations of heavy metals in wild carnivores in Doñana after the Aznalcóllar spill, and the first report on the presence of such elements in Egyptian mongoose and common genet. In all species, levels of contaminants were below those considered high for carnivores according to the work of Wren (1986), Puls (1994), Ma (1996), Cooke and Johnson (1996), Heinz (1996), Thompson (1996), and Mason and Wren (2001).

When compared with the levels described in liver and muscle samples of lynx and otters in Doñana in 1982–83 by Hernández et al. (1985), the levels observed in the present study, after the Aznalcóllar spill, are within the same range as regards Cd, Cu, and Zn and lower as regards Hg and Pb. The sludge released by the Aznalcóllar mine contained high concentrations of Zn (0.8%), Pb (0.8–1.2%), As (0.5–0.6%), and Cu (0.2%) and affected 2754 ha of protected marshes (Grimalt et al., 1999). The carnivores studied here tend to inhabit the Mediterranean woodland surrounding the open marshes, and therefore, although they can feed on prey from the marshes, may not be as exposed as other wild species that feed on plants or soils from the contaminated marshes (Taggart et al., 2005; Mateo et al., 2006).

4.1. *Metal levels in liver in relation to other studies*

Mean liver Zn concentrations detected in this survey were quite constant among species, with means between 124 and 145 µg/g d.w., although maximum levels of 322 µg/g and 307 µg/g were found in one mongoose and one fox. These concentrations are higher than those reported in artic foxes (*Alopex lagopus*) and wolverines (*Gulo gulo*) (Hoekstra et al., 2003), and in American minks and river otters (*Lutra canadensis*) captured on rivers receiving metals discharges in Canada (Harding et al., 1998). However, levels were lower than in otters from Finland (Hyvärinen et al., 2003), and similar to foxes from Switzerland (Dip et al., 2001). This suggests that the observed concentrations of these elements are within the normal reported range for carnivores.

Mean Cd concentrations in liver were similar to those reported in a diverse range of carnivores from Canada who were shown to have levels between 0.10 and 0.30 µg/g d.w. (Harding et al., 1998; Hoekstra et al., 2003), but lower than levels noted in adult otters from Finland (Hyvärinen et al., 2003) and in foxes from Switzerland (Dip et al., 2001). None of these studies reported toxicity associated with such concentrations and the Cd concentrations detected in the present study are at least two orders of magnitude lower than levels that would normally be indicative of chronic intoxication (Beyer, 2000).

Mean Cu concentrations in liver varied widely among species. The mean concentration in fox was 72 µg/g d.w., but five foxes had concentrations between 205 and 950 µg/g. The hepatic Cu liver level found by Mandigers et al. (2004) to be associated with subclinical hepatitis was 419 µg/g d.w. in a study of Cu-sensitive breeds of dog. Also, the concentration observed in fox here is markedly higher than that reported in foxes from Switzerland (Dip et al., 2001). The mean Cu concentration in liver observed in species other than fox in this study was in the range of those reported in artic fox (Hoekstra et al., 2003), minks and otters from Canada (Harding et al., 1998), but, lower than in adult otters from Finland (Hyvärinen et al., 2003) and wolverines from Canada (Hoekstra et al., 2003). Hoekstra et al. (2003) suggested that differences in diet between wolverines and arctic foxes, whereby wolverine eat a high proportion of caribou, may cause the differences

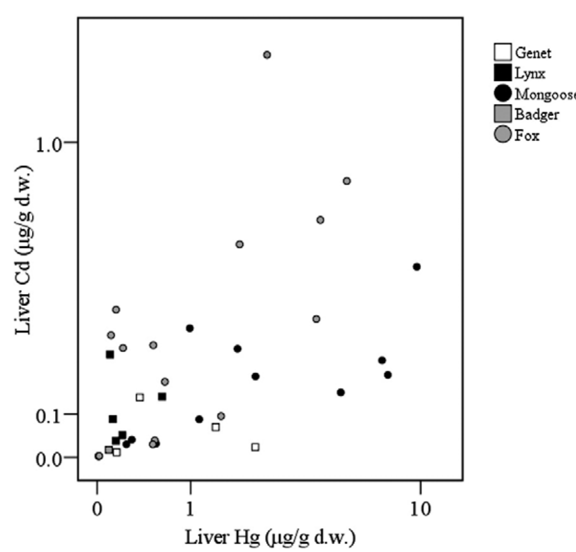

Fig. 4 – Correlation between Hg and Cd concentrations in livers of different species of carnivores. The correlations were significant for the red fox (r=0.831, p<0.001) and the Egyptian mongoose (r=0.806, p=0.003).

noted in these two species from the same habitat. Similarly, foxes from Doñana frequently feed on wild ungulate carrion (Fedriani, 1996), which may explain the higher concentrations of Cu observed in some individuals.

The Hg concentrations detected in this study are below those associated with Hg intoxication to non-marine mammals (30 μg/g w.w.; Thompson, 1996). It would appear that there has been a reduction in the mean concentrations of Hg in livers of carnivores since the 1980s, when Hernández et al. (1985) reported a range of concentrations between 0.25–2.3 μg/g in lynx and 3.9–17.5 in otter, whilst in the present study we found between 0.09 and 1.75 μg/g. This may reflect a reduction in the use of fungicides containing mercury (which are now banned). However, care must be taken when interpreting these results since marked differences may occur between species which simply reflect a greater or lesser exposure to methylmercury, which commonly bioaccumulates in aquatic based food chains (Wren et al., 1996). For example, Hernández et al. (1985) found a mean liver concentration of 8.3 μg/g w.w. in otters (part of an aquatic food web), compared to just 0.76 μg/g in lynx who will predominantly feed on rabbits (*Oryctolagus cuniculus*). The lynx here had the lowest Hg liver concentrations among the studied species, and compared to other wild felids, levels were within the same range as for ocelots (*Felis pardalis*) (Mora et al., 2000) but far lower than seen in bobcats (*Lynx lynx*) (Cumbie, 1975) or Florida panthers (Newman et al., 2004). The highest liver Hg level was reported here for some individual mongoose (max. 9.7 μg/g d.w.). Javan mongooses (*Herpestes javanicus*) from Japan have also shown high levels of liver total Hg (1.75–55.5 μg/g w.w.; Horai et al., 2006).

Liver Pb concentrations observed in this study have also diminished when compared with data from Hernández et al. (1985), who found a mean concentration of 0.22 μg/g w.w. in lynx and 0.19 μg/g w.w. in otters. Our results are also low when compared with concentrations in a diverse range of carnivores from other locations (Dip et al., 2001; Shore et al., 2001; Hoekstra et al., 2003; Alleva et al., 2006), but are in the same range as those reported for minks and otters in Canada (Harding et al., 1998). Importantly, the detected concentrations are well below those detected in incidents of Pb toxicosis in mammals (5–10 μg/g w.w.; Ma, 1996). Interestingly, the bone Pb levels in lynx in the Sierra Morena were significantly higher than in Doñana. This may well reflect a degree of food chain transfer in this area originating from an increased presence of lead minerals in the soils of this area (originating from the mineralised underlying geology), and/or originating from the many abandoned mine workings in this area. Santiago et al. (1998) also observed high Pb levels in samples of wild ungulates in the Sierra Morena area, and reported mean liver concentrations in wild boars (*Sus scrofa*) to be 2.61 μg/g w.w., with a maximum level of up to 43 μg/g.

Mean Se concentrations in liver were similar to those reported in a diverse range of carnivores from Canada (Harding et al., 1998; Hoekstra et al., 2003). Also, as previously observed in a variety of mammals (Prestrud et al., 1994; Woshner et al., 2001; Hoekstra et al., 2003), Se levels were positively correlated with Hg and Cd levels. This may support the hypothesis that there is a functional association between these elements. It has been proposed that Se may form inert metal selenide complexes and/or that the antioxidant activity of selenozyme glutathione peroxidase are utilised to mitigate the toxic effects of Hg and other non-essential heavy metals (Hoekstra et al., 2003).

The observed arsenic levels in liver are slightly lower than those reported in ringed seals (*Phoca hispida*) and polar bears (*Ursus maritimus*) from Alaska (Woshner et al., 2001), but within the range of those observed in dogs from Northern Spain (López-Alonso et al., 2007). In artic foxes and wolverines from Canada, As was found to be below laboratory detection limits in 95% of the samples (Hoekstra et al., 2003).

4.2. *Metal levels in relation to sex, age and species*

Liver concentrations of Se, Cd, Pb and Hg were higher in females than in males. This difference has been previously recorded for kidney Cd in dogs (López-Alonso et al., 2007). In birds, females also often have higher levels of heavy metals than males (Mateo et al., 2001; Burger, 2007). Some of these differences may reflect differential feeding habits between males and females, or different metal dynamics (Gochfeld, 1997). Metallothionein, a metal binding protein, shows some gender differences that may explain the differences in metal accumulation (Nordberg, 1998; Burger, 2007).

Adult red foxes presented higher levels of Cd, Pb, and Hg than cubs in several tissues. Harding et al. (1998) and Hyvärinen et al. (2003) have also observed that Cd accumulated with age in mink and otter, respectively; whilst López-Alonso et al. (2007) observed the most marked increase in kidney Cd in dogs in the early years of life.

The lynx generally presented the lowest levels of most of the metals analyzed. As stated above, whereas the lynx diet comprises 85–90% rabbit (Delibes, 1980), mongoose, genets and especially fox, have more varied feeding habits, which can include birds, reptiles, amphibians, crayfish, eggs or carrion (Palomares and Delibes, 1991; Fedriani, 1996). These differences in diet are likely to provide the main explanation for the resultant differences in metal levels in tissues.

The present study found that the muscle and liver levels of all metals except Pb were positively correlated. This is interesting because, if live carnivores are to be tested for contamination, a biopsy can be made to obtain a muscle sample, as suggested by Lord et al. (2002). These authors believed that muscle biopsies appeared to be the most useful and reliable means of measuring exposure of raccoons to Hg in various environments.

5. Conclusions

Although carnivores are at the top of the food chain, and thus potentially exposed to any biomagnification processes that may occur in a food web, the individuals studied in the present work generally had low levels of heavy metals in their tissues. This is positive in that despite the Aznalcóllar mine spill in Doñana, and the prevailing mineralisation and historic mining activities in Sierra Morena, metals do not appear to represent a major threat for the critically endangered Iberian lynx, or other carnivores. However given the current precarious population status of Iberian lynx, we believe that a robust continuous monitoring process for heavy metal concentrations in conjunction with specific biomarkers of their effect in Iberian lynx and other carnivores remains necessary.

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