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Blood lead levels and δ-ALAD inhibition in nestlings of Eurasian Eagle Owl (*Bubo bubo*) to assess lead exposure associated to an abandoned mining area

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Abstract In order to biomonitor lead contamination in Southeastern Spain, 218 blood samples from 28 to 30-day old Eurasian Eagle Owl chicks (*Bubo bubo*) born between 2003 and 2007 were analysed. In general, mean lead levels showed that chicks were exposed to background concentrations. However, mean levels in chicks born in an ancient and abandoned mining site ("Sierra Minera Cartagena-La Union") or in their surroundings (Geometric mean (GM) = 5.83 μg/dl, range 0.49–25.61 μg/dl), an area highly polluted by lead and other metals, were significantly higher ($p < 0.001$) than the rest of the population (GM = 1.66 μg/dl, range = Non detected–18.37 μg/dl). Because δ-ALAD activity is considered the best biomarker for lead exposure and effect in birds, the activity of this enzyme was also evaluated and correlated with lead levels in blood. In this study, low levels of blood lead inhibited δ-ALAD, even when lead concentrations were lower than the limits described by other authors in raptors. Adverse effects caused by this inhibition may occur when blood lead levels were above 15 μg/dl, although only eight chicks presented these concentrations in their blood. Sampling site also influenced enzymatic activity, since it decreased about 60% in the polluted area in relation to the rest. For all these reasons, further research regarding risk assessment for lead exposure in Eagle Owls nesting in the polluted area is advisable. Our results suggest that the Eurasian Eagle Owl can be considered a suitable sentinel animal for monitoring lead contamination and δ-ALAD activity can be used as a sensitive biomarker for lead exposure and effect in this species.

Keywords Eurasian Eagle Owl · *Bubo bubo* · Lead · δ-ALAD · Biomonitoring · Mining · Blood

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Introduction

The value of birds as biomonitors of environmental pollution has been broadly recognised, and several governmental established monitoring programmes are the proof for it (Becker 2003). Among birds, raptors are considered especially suitable for monitoring bioaccumulable and biomagnificable pollutants, mainly due to their position at the top of trophic chain and susceptibility to bioaccumulate and integrate contaminants over time (Furness 1993). In our study area, the Eurasian Eagle Owl (*Bubo bubo*) can be considered a suitable sentinel species for monitoring persistent environmental pollutants, as it meets the requirements established by the National Research Council (1991).

Mining industry is an important source of lead contamination and several authors have reported significantly higher lead levels is birds inhabiting mining areas than those from unpolluted or reference sites (Beyer et al. 1985, 2004; Eisler 2000; García-Fernández et al. 2005a; Henny et al. 1994). In Southeastern Spain there is an important

population of Eurasian Eagle Owl but some pairs inhabit inside or in the surroundings of an ancient mining site (AMS) located in the mountain "Sierra Minera Cartagena-La Unión". In this site, lead, zinc, copper, tin, iron, manganese and silver had been extracted for more than 2500 years (Pavetti et al. 2006), being the main source for lead and zinc in Spain during the nineteenth century (Estevan-Senís 1967). According to Martínez-Sánchez and Pérez-Sirvent (2009), concentrations of heavy metals in this ancient mining site closed in 1991 are very high, even higher than 1000 mg/kg of lead in soil in some points of the ancient mine, exceeding the limits allowed by several governments (Pavetti et al. 2006). Furthermore, soils in some points of this area are acid, which leads to a high biodisponibility of the metals, especially after rainfalls, because pyrite oxidation transforms sulphurs in sulphates causing solubilisation of heavy metals and increased contamination due to lixiviation (Pavetti et al. 2006).

Lead is considered one of the most toxic heavy metals, causing alterations at vascular, nervous, renal, immune, reproductive, and haematological systems, as well as behavioural abnormalities (Burger 1995; Eisler 2000). Moreover, chronic exposure in birds to this ubiquitous and persistent metal can lead to population declines in more sensitive species (Burger and Gochfeld 2000; Fair and Ricklefs 2002).

Blood sample collection and analyses provide a sensitive and non-lethal means for monitoring health effects and lead body burden (McBride et al. 2004), indicating a recent exposure to this metal (García-Fernández et al. 1997). Direct measurement of contaminants in fluids and tissues can reflect their exposure, absorption and accumulation in organisms; however, monitoring the biological responses would give more direct information about their alterations and toxicological consequences (Vanparys et al. 2008). These responses induced by xenobiotics are called biomarkers and comprise any alteration in cellular or biochemical components or processes, structures or functions, which are measurable in a biological system or sample (National Research Council 1987). At haematological level, lead inhibits the activities of several enzymes necessary for the synthesis of the haeme group in the erythrocyte (δ-aminolevulinic acid dehydratase, ferrochelatase, haeme synthetase, etc.). Because this inhibition is greater on the δ-aminolevulinic acid dehydratase (δ-ALAD) and also the first measurable biochemical change after lead absorption (Tola et al. 1973), it can be used as a biomarker of exposure and effect for lead in birds (Dieter et al. 1976; Scheuhammer 1987a, b). Effects of δ-ALAD inhibition in birds may be more severe than in mammals, since the more metabolically active nucleated red blood cells require porphyrin synthesis not only for haemoglobin production, but also for respiratory haeme containing enzymes (Allen 1971; Brace and Altland 1956). Moreover, consequently to the enzymatic inhibition, there is an accumulation of ALA, its substrate, which causes a neuropathogenic effect, probably by acting as a γ-aminobutiric acid (GABA) receptor agonist in the nervous system (Brennan and Cantrill 1979). Another effect caused by ALA accumulation is a stimulation for reactive oxygen species production (Bechara 1996), which produces lipid peroxidation in the erythrocytes membranes and a subsequent haemolysis (Lawton and Donaldson 1991), which may contribute to the anaemia caused by δ-ALAD inhibition.

Taking into account the possibility of using the Eurasian Eagle Owl as a sentinel species, the aim of our study was to determinate whether blood from nestlings is able to reflect lead exposure associated to environmental pollution in Southeastern Spain, including an area in the range of influence of an ancient and abandoned mining site. Also, inhibition of δ-ALAD activity in blood was used as a biomarker of lead exposure and effect in these Eagle Owl chicks.

Materials and methods

Study area and species of study

The study area comprises the mountains Escalona, Altaona, Monte el Valle, Columbares, Sierra Minera Cartagena-La Union, La Muela-Cabo Tiñoso and Almenara (37°45′N, 0°57′W), in the south of the province of Alicante and the east of Murcia Region, in Southeastern Spain (Fig. 1). The climate is meso-arid Mediterranean with 275–400 mm of annual rainfall and a high average annual temperature of 19°C. The study area is relatively large and some differences in land use are known. Therefore, and for statistical analyses, this area was subdivided in two sub-areas. Northern subarea comprises the mountains Escalona, Altaona, Monte el Valle and Columbares. In this subarea, land is mainly dedicated to citrus and dry farming, and the European Rabbit (*Oryctolagus cuniculus*) is abundant, accounting for 71% of the prey consumed by Eagle Owls (León et al. 2008). In Southern subarea, the European Rabbit is less abundant (35% of the Eagle Owls' diet), and the raptor consumes a similar proportion of rats (*Rattus rattus* and *R. norvergicus*) (23% of the diet), apart from pigeons (*Columba* spp.), partridges (*Alectoris rufa*), hedgehogs (*Erinaceus europeus* and *Atelerix algirus*) and yellow-legged gulls (*Larus michahellis*) (León et al. 2008). This subarea is delimited by Sierra Minera Cartagena-La Union, La Muela-Cabo Tiñoso and Almenara, irrigation farming is predominant and it is remarkable the fact that some ancient mining sites (Sierra Minera de Cartagena-La Unión) are found in this subarea (Fig. 1).

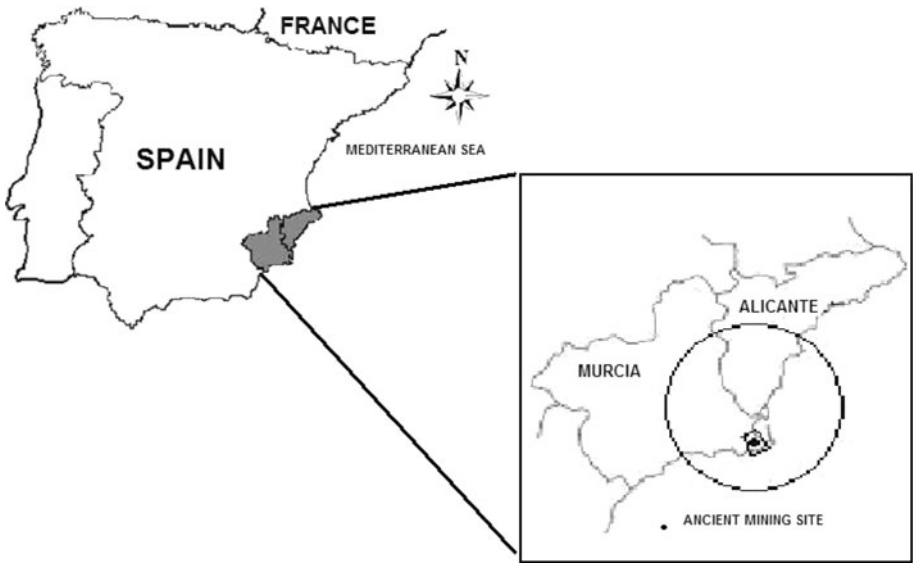

Fig. 1 Province of Alicante and Murcia Region, in Southeastern Spain. Study area is included in the circle and the area under mining influence is delimited in the square

In our study area, the Eagle Owl prefers low to medium cliffs, especially in the Northern subarea. This species is the biggest nocturnal raptor in Spain, where it is sedentary and highly territorial during the whole year, occupying territories which size and foraging area depend on the prey availability. It has been suggested that Eagle Owls hunt mainly in areas of 10–20 km^2 (Haller 1978; Mikkola 1983), although telemetry studies showed that their territories may be larger (Dalbeck et al. 1998). When the habitat is favourable, nests can be relatively close, even at less than 2 km far from each other (Mikkola 1983).

Eagle Owl population in our study area is abundant and has been estimated in approximately 240–270 pairs, some 10–12% of the total population of this species in Spain (Martínez and Calvo 2006; Martínez and Zuberogoitia 2003).

Sample collection

Reproductive activity was rigorously monitored from December until fledging, to determine exact age of the nestlings before blood sampling. In order to avoid stress in the nestlings, careful steps were taken, like covering the head of the nestlings during manipulation. Two hundred and eighteen samples (175 from Northern and 43 from Southern subarea) were obtained from 28 to 30-days old Eagle Owl chicks, between 2003 and 2007 by the veterinarian of the Wildlife Rehabilitation Center "Santa Faz" (Alicante, Spain), who proceeded to evaluate clinically the health status of the chicks prior to blood sampling. Finally, nestlings were returned to the nest. Blood samples from the nestlings (3–5 ml) were taken by puncturing brachial vein with a 23G needle and a syringe. These samples were taken immediately to the laboratory under refrigerated conditions and frozen to −40°C until processing.

Blood lead analyses

Lead levels were analysed in blood samples following the method described by García-Fernández et al. (1995). The samples were prepared for analysis eliminating impurities that might interfere with the results by a complete digestion ensured using high temperature digestion with a mixture of acids following the method described by García-Fernández et al. (1995), in which a volume of 0.2 ml of whole blood was placed in a quartz digestion tube, to which 0.5 ml of an acid mixture (nitric/perchloric/sulfuric, 8:8:1) was added. The sample was then submitted to a progressive thermal treatment and, once dried, was left to cool. Tetradistilled purified water was added and transferred to the measuring vessel, adjusting the final volume to 10 ml.

Prior to by anodic stripping voltammetry (ASV), 50 µl of hydrochloric acid was added to the measuring vessel as an electrolyte support. The pH of the final solution was between 1 and 2. The anodic stripping voltammeter (VA-757 Computrace Workstation; Methrohm, Switzerland) used was equipped with three standard electrodes: working electrode (hanging mercury drop), reference electrode (Ag/AgCl; KCl, 3 mol/l), and auxiliary electrode (platinum). We used the differential pulse normal technique with an electrolysis time of 120 s and modulation amplitude of 50 mV. The concentration of lead in the digested sample was calculated after twice adding dilutions prepared from standard solutions of lead (Sigma, St. Louis, MO). Mean recoveries, which approached 97%, were calculated analysing 10 identical samples spiked with known amounts of lead. To determine the linearity of recovery, European Economic Community (EEC) reference standard for lead (Yeoman et al. 1985) was analysed in triplicate. Reproducibility reached 98.16 ± 1.03% and was determined

analysing 10 identical samples of reconstituted lyophilized blood (European Union Reference Standards CRM195) (García-Fernández et al. 1995). Detection limit was 0.3 μg/dl. All the reagents used were Suprapur quality from Merck (Darmstadt, Germany). The quartz tubes used for the wet digestion were previously washed with 2% nitric acid for 48 h and then rinsed twice with tetradistilled water and dried in an oven at 100°C.

Determination of blood δ-ALAD activity

Enzymatic activity was determined using the method described by Scheuhammer (1987b). A subsample of blood was haemolysed with 4 volume of Triton X-100; then 100 μl of 0.5 M morpholinethanesulfonic acid (MES) and 50 μl of 60 mM aminolevulinic acid (ALA) solution in MES were added to 100 μl of the haemolysate. The samples were incubated at 42°C in a water bath for 1 h and then centrifuged for 5 min at 15,000×*g*. Ehrlich's reagent (dissolve 0.3 g dimethylaminobenzaldehyde in 9 ml glacial acetic acid, add 4.8 ml of 70% perchloric acid, and make up to 15 ml with acetic acid) was added to the supernatant, and using a UV spectrophotometer the absorbance of the samples was read at 555 nm against the appropriate blank. Quantification was based on measuring the colour of the product, porphobilinogen (PBG), that the enzyme forms with the dimethylaminobenzaldehyde. Activity of δ-ALAD is expressed as μmol PBG/h/l red blood cells (RBC).

Statistical analyses

Statistical analysis of the data was performed using SPSS v15.0 statistical software (SPSS Inc., 1989–1999). About fifteen percent of the blood samples analysed showed non-detected values, which were assigned ½ the detection limit established to perform mean comparison tests. Because results of blood δ-ALAD activity and blood lead levels were not normally distributed, values were log-transformed and Pearson's correlation coefficient was applied in order to evaluate the relationship between both parameters. Kruskal–Wallis Test was used to compare lead levels between sub-areas and nests. Level of significance for statistical analyses was set at $\alpha = 0.05$.

Results and discussion

Blood lead levels

Lead concentrations in the samples analysed ranged from non-detected to 25.61 μg/dl. Mean concentration of blood lead levels for the whole population (3.73 ± 4.2 μg/dl) is close to the range considered by several authors for birds non-exposed to lead (García-Fernández et al. 1997; Henny et al. 1991; Martínez-López et al. 2004; Scheuhammer, 1989) and, therefore, similar to the levels found in several raptor species inhabiting non-polluted areas (Henny et al. 1994; Martínez-López et al. 2004; McBride et al. 2004), or even lower if adults were included in other studies (García-Fernández et al. 1997) (Table 1).

However, about 10% of the samples presented blood lead levels above 10 μg/dl, being in eight individuals higher than 14.87 μg/dl. According to Scheuhammer (1989), these 8 chicks could be abnormally exposed to lead. Lead concentrations below 20 μg/dl in Falconiformes were considered by Franson (1996) as background levels. Concentrations above this limit could suggest an exposure to either contaminated food items or ingestion of lead artefacts embed in prey flesh (García-Fernández et al. 2005b; Mateo et al. 1999, 2003; McBride et al. 2004). Only in two samples blood lead levels exceeded this limit, and hunting is forbidden during the breeding season in our study area (Orden de 11 de junio de 2008). For these reasons, ingestion of lead artefacts is an unlikely source of lead for the population studied. Nevertheless, recall that in the Southern subarea some nests (10%) are located in the mountain "Sierra Minera Cartagena-La Unión". In this ancient mining site (AMS), average blood lead concentration of the chicks is 8.61 ± 6.76 μg/dl, higher than in Great Horned Owl (*Bubo virginianus*) nestlings born downstream from an ancient mining site (Table 1) (Henny et al. 1994). However, our results are almost identical to the blood lead levels found in Black Kites (*Milvus migrans*) nestlings (8.4 ± 5.2 μg/dl) born near a solid waste incinerator in Madrid (Spain) (Blanco et al. 2003). These authors suggested that inhalation of lead from dust was the main source for the Black Kites, as lead levels in European Rabbits, the main prey for that species, were too low. Furthermore, a much higher proportion of lead respiratory intake may be retained and absorbed compared with ingested lead (Ma 1996; Tejedor and González 1992). However, dietary intake can not be ruled out in our study and prey analyses would be also needed. As mentioned above, European Rabbit is less abundant in Southern subarea than in the Northern (León et al. 2008) and prey items other than European Rabbit would be expected to contain higher lead levels due to their different feeding habits (Vermeulen et al. 2009). Nevertheless, lead levels in the polluted area were still significantly higher than in the rest of the Southern subarea ($p = 0.013$). Considering that birds that spend significant amounts of time feeding in environments highly contaminated with lead (e.g., near metal mines) can be exposed to lead in the absence of lead shot ingestion (Benson et al. 1976), and that 22% of the chicks born in the AMS presented lead levels above 15 μg/dl, we could suggest that especial effort should be taken for

Table 1 Lead blood levels and δ-ALAD activity in blood of raptors inhabiting polluted

Species	Reference		Blood lead levels (μg/dl)		δ-ALAD activity	
			Polluted area	Non-polluted area	Polluted area	Non-polluted area
Great horned owl nestlings (*Bubo virginianus*)	Henny et al. (1994)	Mean	3.8	–	219.7[a]	–
		Min–max	<5–14		93–346[a]	
		(*n*)	(10)		(10)	
Northern harrier nestlings (*Circus cyaneus*)	Henny et al. (1994)	Mean	6.7	4.2	399[a]	612.6[a]
		Min–max	<3.8–67.5	<1.4–14.1	174–600[a]	529–671[a]
		(*n*)	(41)	(8)	(41)	(8)
American kestrel nestlings (*Falco sparverius*)	Henny et al. (1994)	Mean	24	8.7	256.6[a]	566.1[a]
		Min–max	<5–227	<3.4–35	51–393[a]	383–724[a]
		(*n*)	(30)	(22)	(30)	(22)
Red-tailed hawk nestlings (*Buteo jamaicensis*)	Henny et al. (1994)	Mean	<5	<5	188.6[a]	323.5[a]
		Min–max	–	–	165–313[a]	294–353[a]
		(*n*)	(2)	(2)	(2)	(2)
Western Screech-Owl nestlings (*Megascops kennicottii*)	Henny et al. (1994)	Mean	10	–	473.3[a]	–
		Min–max	<5–71		469–478[a]	
		(*n*)	(3)		(3)	
Eagle Owl (*Bubo bubo*)	García-Fernández et al. (1997)	Mean ± SD	–	7.6 ± 2	–	–
		Min–max		6–20		
		(*n*)		(7)		
European Kestrel (*Falco tinnunculus*)	García-Fernández et al. (1997)	Mean ± SD	–	10 ± 1.3	–	–
		Min–max		5–26		
		(*n*)		(12)		
European Buzzard (*Buteo buteo*)	García-Fernández et al. (1997)	Mean ± SD	–	10.8 ± 1.5	–	–
		Min–max		3–17		
		(*n*)		(5)		
Little Owl (*Athene noctua*)	García-Fernández et al. (1997)	Mean ± SD	–	9.4 ± 2		
		Min–max		6–15		
		(*n*)		(5)		
Booted eagle nestlings (*Hieraaetus pennatus*)	Martínez-López et al. (2004)	Mean ± SD	–	3.21 ± 1.98	–	679.1 ± 67[b]
		Min–max		1.10–10.57		537.5–849.1[b]
		(*n*)		(27)		(18)
European buzzard nestlings (*Buteo buteo*)	Martínez-López et al. (2004)	Mean ± SD	–	2.74 ± 1.04	–	744.2 ± 87.9[b]
		Min–max		1.34–3.54		650–852[b]
		(*n*)		(4)		(4)
Goshawk nestlings (*Accipiter gentilis*)	Martínez-López et al. (2004)	Mean ± SD	–	7.60 ± 3.64	–	584 ± 51.7[b]
		Min–max		3.93–11.21		539–640.5[b]
		(*n*)		(3)		(3)
Cooper's Hawk juveniles (*Accipiter cooperii*)	McBride et al. (2004)	Mean ± SD		0.28 ± 0.04		74.9 ± 2.2[c]
		Min–max		ND–0.71		67.2–84.2[c]
		(*n*)		(15)		(*n* = 9)
Eagle Owl nestlings (*Bubo bubo*)	This study	Mean ± SD (GM)	8.61 ± 6.76 (5.83)	3.18 ± 3.43 (1.66)	65.17 ± 50.19 7.97–203.11	165.83 ± 117.31 3.98–661.12
		Min–max	0.49–25.61	ND–18.37	(22)	(196)
		(*n*)	(22)	(196)		

ND non detected values, *SD* standard deviation, *GM* geometric mean

[a] Unit activity is defined as an increase in absorbance of 0.100 at 555 nm with 1.0-cm light path/ml erythrocytes/h at 38°C

[b] μmol PBG/h/l RBC

[c] nmol ALA/min/ml RBC

monitoring this area in order to evaluate whether Eagle Owls breeding in these highly polluted territories could be at risk for heavy metals exposure along their lifetime.

Differences in mean blood lead levels between nests and subareas are significant ($p < 0.001$), although differences in mean levels among subareas (3.05 ± 3.4 μg/dl in the Northern subarea and 6.5 ± 5.9 μg/dl in the Southern subarea) are due to the AMS chicks. When we exclude the samples obtained in the AMS, mean blood lead levels results in 3.18 ± 3.43 μg/dl (Table 1), significantly lower than the mean levels found in AMS ($p < 0.001$) (Fig. 2) and very similar to those found in Booted Eagle (*Hieraaetus pennatus*) and European Buzzard (*Buteo buteo*) nestlings inhabiting a non-polluted area in the Murcia Region (Southeastern Spain) (Martínez-López et al. 2004) (Table 1). These low levels were expected in our study because most of the nests are, in principle, located far from potential sources for heavy metals. Exposure to lead in these nestlings may be due to the ubiquity of lead in the environment, by continued exposure by ingestion or inhalation. Heavy metals in developing embryos and nestlings after hatching may also derive from egg content. However, several authors have suggested that very little lead is transferred from female body to eggs (Furness 1993) and the contribution of metals sequestered in eggs has been recorded to be minimal compared to that from nestling exposure since hatching to fledgling in Herring Gulls (*Larus argentatus*) (Burger and Gochfeld 1997) and in Black Kites nestlings breeding near a solid-waste incinerator in Madrid (Spain) (Blanco et al. 2003).

δ-ALAD activity and correlations between lead and δ-ALAD in blood

Average values of δ-ALAD activity in blood were 155.67 ± 116.33 μmol PBG/h/l RBC. The knowledge of the basal activity of δ-ALAD in different species and at different moments of the birds' life cycle is still scarce and our results are not always comparable with data found in literature (Table 1) because enzymatic activity has been analysed with different techniques. However, differences in enzyme inhibition regarding polluted or unpolluted areas coincide with our samples and the existence of negative correlations with blood lead levels may be sufficient to indicate possible sublethal effects. In our study, this negative correlation was found ($P = -0.137$, $p = 0.044$) (Fig. 3), showing a more significant relation when blood lead levels were higher than 4 μg/dl ($P = -0.341$, $p = 0.006$). These results show an inhibition of the enzyme activity, which reaches a 55% when lead concentrations in blood exceed 15 μg/dl. However, as shown in Fig. 3, lower concentrations of lead in blood also cause enzymatic inhibition, even below the limit established by Henny et al.

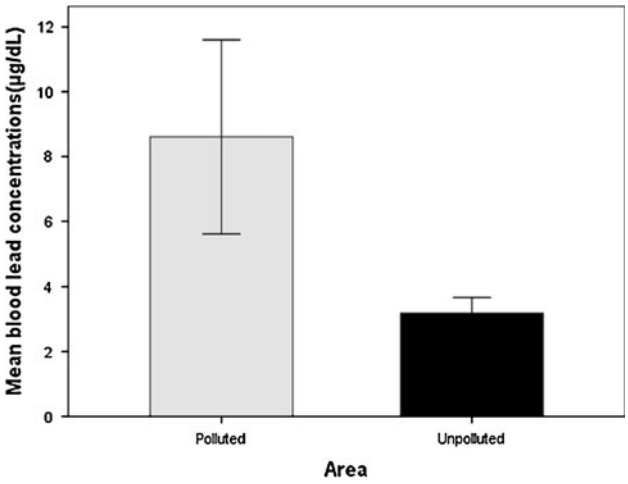

Fig. 2 Mean blood lead concentrations (μg/dl) in Eurasian Eagle Owl nestlings from the polluted (Ancient Mine Site) and the unpolluted area (IC: 95%) ($p < 0.001$)

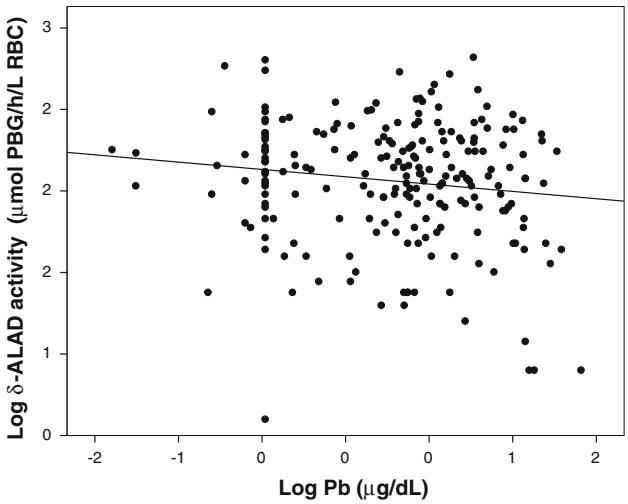

Fig. 3 Relationship between lead blood levels (μg/dl) and δ-ALAD activity in Eurasian Eagle Owl nestlings blood ($P = -0.137$, $p = 0.044$)

(1994) in American Kestrels (*Falco sparverius*) and by Martínez-López et al. (2004) in Booted eagle, Goshawk (*Accipiter gentilis*) and European Buzzard. It is remarkable that a 31% of enzymatic inhibition was still found when blood lead levels exceeded 10 μg/dl. Sampling site also influenced enzymatic activity, since it decreased about 60% in the polluted area in relation to the non-polluted.

Enzymatic inhibition induced by lead can differ between species, age and even physiological status. Consequent pathologic effects can therefore be also different. In relation to this, Beyer et al. (1988) found differences in δ-ALAD inhibition by lead in six bird species and studies have shown that lead is considerably more toxic to nestlings of altricial species like American Kestrel, than for hatchlings of

precocial species which include Chickens (*Gallus gallus*), Japanese quail (*Coturnix coturnix*), Mallards (*Anas platyrhynchos*) and Pheasants (*Phasianus colchicus*) (Hoffman et al. 1985a). Adult birds or precocial hatchlings need a continued 80% or greater blood δ-ALAD inhibition for decrease haemoglobin after lead exposure (Eastin et al. 1983; Franson and Custer 1982; Hoffman et al. 1981; Pain and Rattner 1988), while only a 45–59% decrease in δ-ALAD is sufficient in American Kestrel nestlings in laboratory and field studies to cause anaemia by significant declines in haemoglobin and haematocrit (Henny et al. 1994; Hoffman et al. 1985b). These interspecific variations are also suggested between raptors regarding the relationship between δ-ALAD depression and anaemia (Franson et al. 1983). According to these studies, it is plausible that some individuals from our population could suffer from anaemia due to δ-ALAD inhibition. Moreover, behavioural deficits and growth retardation has been evidenced in Herring Gulls with blood lead levels close to the highest found in our population (Burger and Gochfeld, 1996). However, further research in the species under study is needed.

Conclusion

According to the concentrations of lead in blood, Eurasian Eagle Owl chicks from Southeastern Spain are exposed to background concentrations. However, 10% of this population was born inside or in the surroundings of the ancient and abandoned mining site (AMS) "Sierra Minera Cartagena-La Union", where mean blood lead levels are significantly higher than the rest of the population. The negative correlation found between δ-ALAD and lead suggests that the activity of this enzyme in blood of Eurasian Eagle Owl nestlings can be used as a sensitive biomarker for lead exposure and effect in this species, even when blood lead levels are below the limit described by other authors as the lowest for δ-ALAD activity inhibition by lead. In relation to our findings, further research regarding risk assessment for lead exposure in Eagle Owls nesting in the AMS could be necessary.

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