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Biomonitoring of heavy metals (Cd, Hg, and Pb) and metalloid (As) with the Portuguese common buzzard (*Buteo buteo*)

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Abstract The accumulation of heavy metals in the environment may have a wide range of health effects on animals and humans. Thus, in this study, the concentrations of arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in the blood and tissues (liver and kidney) of Portuguese common buzzards (*Buteo buteo*) were determined by inductively coupled plasma-mass spectrometer (ICP-MS) in order to monitor environmental pollution to these elements. In general, Hg and As were the elements which appeared in the highest and lowest concentrations, respectively. A highest percentage of non-detected concentration was found for blood Cd (94.6 %) but, in turn, it was the only metal that was detected in all kidney samples. The kidney was the analyzed sample which showed the highest concentrations of each element evaluated. Statistically, significant differences among blood, liver, and kidney samples were observed for As and Cd ($P<0.05$). Cd concentrations in kidney and liver varied significantly with age: Adults showed higher hepatic and renal Cd concentrations than juveniles. Blood Pb concentration seems to show an association with the hunting season. Although raptors are at the top of the food chain and are thus potentially exposed to any biomagnification processes

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that may occur in a food web, the individuals evaluated in this study generally had low levels of heavy metals in blood and tissues. However, chronic exposure to these metals was verified. The results presented here lend weight to arguments in favor of continuous biomonitoring of metals and metalloids, since heavy metals may accumulate to levels that will pose a risk to both human health and the environment.

Keywords *Buteo buteo* · Common buzzard · Heavy metals · Metalloid · Raptors · Portugal

Introduction

Trace elements are present in the environment through the geological cycle and various anthropogenic activities, with the latter the most relevant (Licata et al. 2010; Naccari et al. 2009). These elements can easily enter the food chain, and at high doses they can be acutely lethal, while at lower doses they may have a wide range of health effects—such as mutagenicity, carcinogenicity, teratogenicity, inmunosuppression, poor body condition, and impaired reproduction in humans and animals (Florea and Busselberg 2006; Scheuhammer 1987). All of which make them a serious threat to the stability of ecosystems and living organisms (Battaglia et al. 2005; López-Alonso et al. 2007; Naccari et al. 2009).

Levels of trace elements and their effects on organisms are influenced by numerous factors related to habitat, physiology, and life history (Peakall and Burger 2003). Exposure to nonessential elements generally is below levels thought to be acutely toxic (Henny et al. 1995; Stout and Trust 2002; Wayland et al. 2001b). Although acute toxicity is unlikely to occur, chronic exposure to nonessential elements may interact with other environmental stressors namely parasitic infections or other pathogens and this could compromise bird's survival and reproduction (Wayland et al. 2008). It should be appreciated that potentially sublethal effects caused by chronic exposure to environmental contaminants are largely unknown in wild birds (Shlosberg et al. 2011).

Biomonitoring of trace elements in the environment has enabled the identification of many sources of pollutants. Typically, the bioavailability of environmental pollutants is assessed by measuring chemical residues in tissues or fluids taken from animals living in specific habitats (López-Alonso et al. 2007). The direct measurement of contaminants in blood and internal tissues is the best indicator of the degree and type of exposure to them (García-Fernández et al. 1997), presenting blood the advantage of being easily accessible, sampling can be relatively harmless, and it is in contact with all tissues where chemicals are deposited and stored (Esteban and Castano 2009). Some species have biological habits that increase the likelihood of exposure to contaminants and can produce relevant information that would be missed if only the areas water or soil were analyzed (Battaglia et al. 2005). However, assessing an ecosystem's health adequately by means of biomonitoring requires the selection of species that are representative. Territorial birds of prey, ones that are non-migratory and have long life spans, are likely to reflect chemical contamination within their extended home ranges (Pérez-López et al. 2008). These localised, upper-trophic level species are also believed to be especially vulnerable to metals and play a very important role as environmental-contamination indicators (Stout and Trust 2002; Wayland et al. 1999).

The common buzzard (*Buteo buteo*), a diurnal bird of prey belonging to the order Accipitriformes and to the family Accipitridae, was the sentinel species selected for this study for several reasons: it is abundant within Portuguese territory and the Portuguese population of these birds is essentially resident, though in autumn and winter a comparatively small number of common buzzards from northern Europe do reach the Iberian Peninsula. What is more, these birds are very territorial, are present in different habitats (forests, agricultural zones, mountain regions, and sub-urban areas), they feed on a wide range of prey, and are very opportunistic hunters (Catry et al. 2010).

The present study was carried out in order to evaluate the degree and type of exposure to trace elements that the Portuguese common buzzards may be exposed and to monitor environmental pollution. For this purpose, we determine the arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) concentrations in whole blood, liver, and kidney samples taken from common buzzards from different areas of Portugal. Also, differences between their areas of origin and the influence of age and gender on toxic-metal concentrations were studied. The influence of the season the samples were taken in, on blood-metal concentrations, was also investigated.

Material and methods

Sample collection

All samples collected from common buzzards came from five Portuguese wildlife rehabilitation centers (*Centro de Ecologia*, *Recuperação e Vigilância de Animais Selvagens* (CERVAS); *Centro de Recuperação de Animais Selvagens do Parque Nacional da Peneda do Gerês*; *Centro de Recuperação de Animais Selvagens do Parque Biológico de Gaia*; *Centro de Recuperação e Investigação de Animais Selvagens da Ria Formosa* (RIAS); and *Centro de Estudos e Recuperação de Animais Selvagens de Castelo Branco* (CERAS)) between November 2007 and January 2012.

Collected animals were either found dead or brought to the centers alive but injured or debilitated due to several potential reasons. These reasons were as follows: collision with a vehicle (n=18), collision with power lines (n=17), lead shot (n=22), fall from nest (n=14), malnutrition (n=3), and injury of unknown origin (n=51). The following data were registered for each bird: date of arrival at the center, area of origin, reason for being brought in, gender (male, female, or unknown), and age (juvenile, adult, or unknown). The birds' origin was divided into two different Portuguese regions: littoral (urban and industrial areas) and up-country (rural and natural areas).

From a total of 125 common buzzards, blood (n=93), liver (n=56), and kidney (n=36) samples were collected. The number of samples collected across different years, seasons, regions, and for different gender and age classes were listed in Table 1. Blood samples were collected via the brachial vein at the moment of arrival to the rehabilitation center and immediately transferred into collection tubes without the use of an anticoagulant. Liver and kidney samples were collected from animals that were found dead, died of natural causes, or were sacrificed when their state of health indicated a potential recovery was unlikely. Liver and kidney samples were placed individually in plastic bags. In this study, only samples from animals that died in the rehabilitation center within the first month after admission were included. All samples were stored at −20ºC until analysis.

Table 1 Data relating to common buzzards (*Buteo buteo*) of this study

		Blood (n=93)	Liver (n=56)	Kidney (n=36)
Year	2007	5	0	0
	2008	8	6	0
	2009	24	19	5
	2010	33	24	24
	2011	22	7	7
	2012	1	0	0
Season	Spring	15	11	6
	Summer	27	15	7
	Autumn	34	17	15
	Winter	17	13	8
Origin	Littoral	47	28	24
	Up-country	46	28	12
Age	Adult	30	19	16
	Juvenile	44	24	9
	Unknown	19	13	11
Sex	Female	21	15	8
	Male	35	26	19
	Unknown	27	15	9

Analytical procedure

Sample analysis was performed in the laboratories of the Scientific-Technical Services of the University of Barcelona (SCT-UB), Spain. Liver and kidney subsamples [250–350 mg wet weight (w.w.)] were digested in Teflon reactors with 3 ml of 65 % concentrated nitric acid (HNO_3) and 2 ml of 30 % hydrogen peroxide (H_2O_2) at 90ºC in an oven and left overnight. According to the volume of blood contained in the tubes, different amounts of HNO_3 and H_2O_2 were used until the digestion of blood samples was complete. After digestion, each liver and kidney sample was brought up to a volume of 40 ml with milli-Q water and according to the blood volume to be analyzed, blood samples were brought up to a volume of 20, 30, or 40 ml with milli-Q water. All samples were transferred to the measuring vessel and then analyzed for As, Cd, Hg, and Pb in an inductively coupled plasma-mass spectrometer (ICP-MS) (Perkin Elmer Model Elan 6000, Perkin Elmer, Waltham, USA). All material used in the digestion process was thoroughly acid-rinsed. A second set of identical liver and kidney samples (1–2 g) was oven-dried at 60ºC until reaching a constant weight in order to calculate the percentage of humidity in each sample, which enabled the transformation of wet-weight results into dry-weight (d.w.) values (Ribeiro et al. 2009).

An analytical quality-control program was applied throughout the study, according to López-Alonso et al. (2007). Blank absorbance values were monitored throughout the survey and were subtracted from the readings before the results were calculated. The limits of detection (LOD) in the acid digest (set at three times the standard deviation of the reagent blanks) were in all cases <0.5 μg/l, and the limits of quantification (LOQ), expressed as a concentration in the blood and tissue, were calculated on the basis of the mean sample weight and volume analyzed. Analytical recoveries were determined from the certified standard reference materials (Whole-blood Seronorm, Trace Element, Whole Blood 2 ref. 201605, and Whole Blood 3 ref. 102405 from SERO AS, Norway and Bovine Liver-1577b from National Institute of Standards and Technology, Gaithersburg, USA) analyzed together with the samples. The range of recovery rates (in view of the concentrations in the reference material) ranged between 93.67 % for hepatic Pb and 138.43 % for blood As.

Data analysis

Statistical analyses were performed with IBM SPSS Statistics for Windows, V.19.0.

A statistical significance level of $P<0.05$ was used for null hypothesis rejection.

Each sample below the LOQ was assigned a value of one-half the LOQ and included in the data set for statistical treatment, a technique which minimizes nominal type I error rates (Clarke 1998).

Normal-distribution assumption was checked using the Kolmogorov–Smirnov test. When normal distribution assumption was violated, the data sets were log-transformed before analysis and checked with the Kolmogorov–Smirnov test. However, most of the variables did not follow normal distribution even after transformation, so a non-parametric approach to the data analysis was necessary. The Mann–Whitney *U* test was used to test the statistical significance for area of origin, age, and gender in the blood and tissues concentrations. Birds with unknown age and gender were not included in the statistical analysis when testing the significance for these variables. Comparisons across the different seasons in terms of blood concentrations and differences between metal concentrations in the blood, liver, and kidney samples were tested using the Kruskal–Wallis test followed by the Dunn's post-hoc test. A non-parametric Spearman`s test was applied to test the correlation between blood and tissues and between tissue concentrations for each analyzed metal.

Results

Heavy metals and metalloid concentrations found in the blood, liver, and kidney of common buzzards are listed in Table 2.

Concerning As concentrations, blood As was not detected in 30.1 % of total samples, in the liver and kidney samples it was not detected in 37.5 % and 19.4 %, respectively (Table 2). Mean As concentrations were significantly statistically different between animals' blood, liver, and kidney ($P<0.01$): the lowest mean concentration was found in blood (0.014± 0.014 μg/g w.w) and the highest in the kidney (0.041± 0.026 μg/g w.w.) (Table 2 and Fig. 1). As concentrations in kidney samples varied significantly with age and gender: Adults showed higher ($P<0.05$) renal-As concentrations than juveniles and females showed renal-As concentrations that were nearly twice as high as average concentrations in males ($P<0.05$). No significant influence from any factor was detected in blood- and hepatic-As concentrations (Table 3).

When considering Cd results, this was not detected in 94.6 % of total blood samples, and for that reason, the influence of age, gender, origin, and season on concentrations of this metal in blood was not studied. In contrast, Cd was detected in 96.4 % of liver samples and in all the kidney samples (Table 2). Mean Cd concentrations were significantly (nearly four times) higher ($P<0.05$) in the kidney (0.373±0.381 μg/g w.w.) than in the liver (0.089±0.097 μg/g w.w.) (Fig. 1). Cd concentrations in tissues varied significantly with age: Adults showed higher hepatic- (twice as high, $P<0.01$) and renal- (three times as high, $P<0.05$) Cd concentrations than juveniles. No significant influence of origin and gender in hepatic and renal levels were detected for Cd accumulation (Table 3).

In this study, Pb was detected in most of the samples: 97.8 % (blood), 87.5 % (liver), and 94.4 % (kidney) (Table 2). Mean Pb concentrations were very similar between blood (0.142±0.628 μg/g w.w.) and liver (0.152±0.194 μg/g w.w.). In the kidney, the highest mean Pb concentration (0.245±0.364 μg/g w.w.) was found, but this difference was not statistically significant (Fig. 1). Blood Pb was significantly affected by age

Table 2 Arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) concentrations in the blood, liver, and kidney of common buzzards from Portugal analyzed in the present study

	Blood (*n*=93)		Liver (*n*=56)		Kidney (*n*=36)	
	μg/dl	μg/g w.w.	μg/g d.w.	μg/g w.w.	μ/g d.w.	μg/g w.w
As						
Mean±S.D	1.489±1.457	0.014±0.014	0.104±0.136	0.029±0.039	0.180±0.133	0.041±0.026
Median	1.391	0.013	0.074	0.022	0.149	0.036
Minimum	ND	ND	ND	ND	ND	ND
Maximum	8.508	0.082	0.978	0.281	0.588	0.112
n<LOQ/%	28/30.1 %		21/37.5 %		7/19.4 %	
Cd						
Mean±S.D	0.201±0.567	0.002±0.005	0.322±0.361	0.089±0.097	1.553±1.706	0.373±0.381
Median	0.102	0.001	0.184	0.050	0.865	0.216
Minimum	ND	ND	ND	ND	0.033	0.009
Maximum	4.447	0.043	1.801	0.450	8.344	1.697
n<LOQ/%	88/94.6 %		2/3.6 %		0	
Pb						
Mean±S.D	14.711±65.156	0.142±0.628	0.541±0.687	0.152±0.194	0.945±1.356	0.245±0.364
Median	5.864	0.056	0.284	0.079	0.443	0.102
Minimum	ND	ND	ND	ND	ND	ND
Maximum	631.473	6.089	3.468	0.949	5.331	1.386
n<LOQ/%	2/2.2 %		7/12.5 %		2/5.6 %	
Hg						
Mean±S.D	20.940±26.728	0.202±0.258	1.387±1.242	0.389±0.346	2.086±1.689	0.503±0.310
Median	12.603	0.121	1.168	0.319	1.850	0.448
Minimum	ND	ND	ND	ND	ND	ND
Maximum	164.895	1.590	5.314	1.479	5.945	1.482
n<LOQ/%	11/11.8 %		3/5.4 %		1/2.8 %	

S.D. standard deviation, *n* number of samples, *LOQ* limit of quantification, *ND* non-detected

($P<0.01$), adults had higher concentrations than juveniles, and by season ($P<0.01$), blood samples collected in autumn had higher Pb concentrations than those collected in spring and summer. Hepatic and renal Pb concentrations were not significantly affected by age, gender, and origin (Table 3).

Turning to Hg results, this metal was also detected in most of the samples: 88.2 % (blood), 94.6 % (liver), and 97.2 % (kidney) (Table 2). Blood Hg concentrations were significantly lower than hepatic and renal Hg ($P<0.001$). Hg accumulation was mainly in the kidney, although there is no significant difference between hepatic and renal concentrations (Fig. 1). Blood Hg concentrations were significantly higher ($P<0.01$) in adults than in juveniles. The season the samples were taken in also had significant effects on blood Hg concentrations ($P<0.001$): Higher levels were found in autumn and winter than in spring and summer, but significant differences were only verified between autumn and spring and autumn and summer. Hepatic and renal Hg was not significantly affected by any of the variation factors considered in this study (Table 3).

The existence of a statistical relationship between metal concentrations in blood and in the different tissues was studied by means of simple correlation coefficients (Table 4). The relationship between Cd contents in blood and tissues was not studied, since it was barely detected in blood. The highest correlation was observed between hepatic and renal Hg concentrations ($R=0.946$, $P<0.001$).

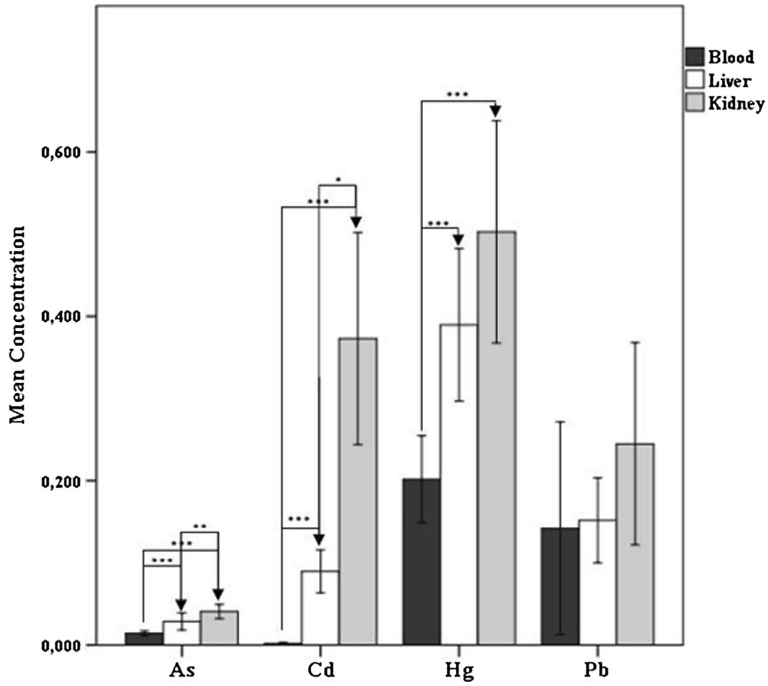

Fig. 1 Bar chart showing the toxic metal concentrations (expressed as arithmetic means, µg/g wet weight) in the blood, liver, and kidney in the 125 common buzzards considered in this study. *$P<0.05$, **$P<0.01$, ***$P<0.001$

Discussion

Previous data on the concentrations of nonessential elements in the blood and tissues of common buzzard are scarce. In fact, this study reports the first data on the concentrations of trace elements (As, Cd, Hg, and Pb) in blood and tissues of raptors residing in Portugal.

The common buzzards, being birds of prey, are at the top of the food chain and consequently are potentially exposed to any biomagnification processes that may occur in a food web (Gochfeld and Burger 1987). In the common buzzard, concentrations of toxic elements reported in the literature were usually determined in the liver and kidney (Battaglia et al. 2005; Castro et al. 2011; Naccari et al. 2009; Pérez-López et al. 2008). Concentrations of heavy metals in the liver and kidney can be considered suggestive of chronic exposure to metals while concentrations in blood reflect recent exposure and thus can be used as a real-time monitor for all stages of the birds' life-cycles. Better understanding of the relationship between heavy-metal concentrations in tissues and blood would enable the researchers to assess whether high concentrations in blood warrant concern regarding their toxic effects, without having to use lethal sampling techniques (Burger and Gochfeld 1997; Castro et al. 2011; Naccari et al. 2009; Wayland et al. 2001a).

There have been a few studies on the transfer of As through terrestrial food chains to predatory birds and on the presence of such metalloid in the raptor tissues, but it is known that vertebrate top predators experiencing higher As burdens (Erry et al. 1999; Lebedeva 1997; Lucia et al. 2012b). In fact, published data on arsenic levels in common buzzards is sparse (Naccari et al. 2009; Pérez-López et al. 2008), and this study reports the first data on blood As concentrations in this species, so it is difficult to compare our results with other studies. However, other species of birds sampled in natural areas (Burger and Gochfeld 1997) and near potential sources of metals (Baos et al. 2006a, b; Blanco et al. 2003) showed similar and higher blood As concentrations, respectively. With respect to As concentrations in the liver and kidney, our results are much lower than those observed for the common buzzard in Galicia, Spain (Pérez-López et al. 2008) and in Sicily, Italy (Naccari et al. 2009) where the exposure to As was considered of no toxicological concern. However, we obtained similar or slightly lower concentrations than those obtained by Erry et al. (1999) in kestrels (*Falco tinnunculus*) from Britain, which have similar feeding habits to common buzzards. Erry et al. (1999) also measured hepatic and renal As concentrations in barn owls (*Tyto alba*) that despite having similar feeding habits with kestrels and common buzzards, presented lower As concentrations

Table 3 Heavy metals in adults and juveniles birds, females and male birds, from littoral and up-country birds and in blood samples collected in the different seasons expressed as mean value (M.V.)±standard deviation (S.D.) μg/dl in blood and μg/g d.w. in tissues

Sample			As M.V±S.D.	Cd M.V±S.D.	Pb M.V.±S.D.	Hg M.V.±S.D.
Blood (μg/dl)	Age	Adult	1.578±1.178	–	9.865±8.302[a]	20.853±13.770[b]
		Juvenile	1.451±1.647	–	5.704±7.534[a]	14.856±20.964[b]
	Gender	Female	1.698±1.188	–	5.645±8.000	14.688±14.085
		Male	1.662±1.734	–	6.291±5.692	22.642±26.565
	Origin	Littoral	1.389±1.088	–	20.852±91.300	27.605±34.075
		Up-country	1.593±1.769	–	8.436±8.860	14.131±13.409
	Season	Spring	2.267±2.220	–	5.413±6.493[c]	11.871±15.363[d]
		Summer	1.297±1.249	–	6.310±9.278[c]	8.191±9.571[d]
		Autumn	1.438±1.271	–	9.608±7.353[c]	30.268±28.510[d]
		Winter	1.205±1.265	–	9.899±8.560	29.429±38.931
Liver (μg/g d.w.)	Age	Adult	0.104±0.055	0.460±0.454[e]	0.443±0.433	1.481±1.330
		Juvenile	0.118±0.192	0.209±0.278[e]	0.441±0.618	1.130±1.132
	Gender	Female	0.096±0.048	0.199±0.196	0.323±0.348	1.364±1.341
		Male	0.083±0.058	0.342±0.405	0.508±0.555	1.415±1.385
	Origin	Littoral	0.077±0.044	0.273±0.359	0.484±0.519	1.618±1.489
		Up-country	0.132±0.184	0.371±0.364	0.596±0.829	1.155±0.903
Kidney (μg/g d.w.)	Age	Adult	0.217±0.139[f]	2.165±2.162[g]	0.828±1.326	1.895±1.725
		Juvenile	0.139±0.068[f]	0.698±0.975[g]	0.822±1.558	1.816±1.331
	Gender	Female	0.245±0.106[h]	2.056±1.793	0.706±0.762	2.407±1.705
		Male	0.166±0.131[h]	1.496±1.990	0.892±1.533	1.745±1.720
	Origin	Littoral	0.150±0.092	1.620±1.972	0.874±1.207	2.455±1.871
		Up-country	0.241±0.179	1.421±1.048	1.087±1.665	1.348±0.928

[a] (Z=−2.940, P<0.003), [b] (Z=−3.164, P<0.002), [c] (H=11.639, P<0.008), [d] (H=24.190, P<0.001), [e] (Z=−2.641, P<0.008), [f] (Z=−2.040, P<0.043), [g] (Z=−1.981, P<0.048), [h] (Z=−2.447, P<0.013)

than those obtained in these two species. Taking into account this previous information, the study of common buzzards in Portugal showed a low level of exposure to this metalloid. However, the significantly higher As levels found in kidney samples of adult birds suggest that As is being bioconcentrated over time. This mean that common buzzards face chronic exposure to this metalloid (Eisler 1998). Despite the As burdens detected in common buzzards were not likely to have been associated with adverse health effects (this element could not be considered as a threat for the survival of the studied birds), it could be involved in sublethal effects (Lucia et al. 2012a, b).

Table 4 Correlations coefficients (R) between blood and tissues and between tissues for each element in common buzzard

	Blood*Liver (n=24)	Blood*Kidney (n=15)	Liver*Kidney (n=36)
As	R=0.572**	R=0.530*	R=0.886***
Cd	–	–	R=0.831***
Pb	R=0.859***	R=0.875***	R=0.811***
Hg	R=0.857***	R=0.832***	R=0.946***

[*] P<0.05, [**] P<0.01, [***] P<0.001

Cd has been described as one of the most dangerous trace elements from environmental and toxicological standpoints, both for humans and animals, not only for its high toxicity but also for its persistence (Battaglia et al. 2005; García-Fernández et al. 1996; Licata et al. 2010). Cd distribution and bioaccumulation patterns observed in our study are consistent with the previous studies in the common buzzard (Battaglia et al. 2005; Castro et al. 2011; García-Fernández et al. 1996; Jager et al. 1996; Naccari et al. 2009). The kidney was the organ with the highest concentration. In contrast, Cd in the blood was not detected in 94.6 % of blood samples, which reflect its low concentrations in blood and/or may be associated with the detection limit of our analytical method. In the blood samples where Cd was detected, the mean concentration was higher than that obtained by García-Fernández et al. (1996) in samples of whole blood from wild birds. Comparison of the Cd concentrations in tissues obtained in this study with those obtained in other recent studies with the same species reveals that our results differ depending on the geographical area: similar with the results obtained in Italy by Alleva et al. (2006), Battaglia et al. (2005), and Naccari et al. (2009), lower than those observed in Galicia, Spain by Pérez-López et al. (2008) and Castro et al. (2011), and higher than the results obtained by García-Fernández et al. (1995) in Murcia, Spain. According to Scheuhammer (1987), the hepatic and renal-Cd concentrations obtained in our study are indicative of a prolonged exposure to low and background amounts of this metal.

Pb is a highly toxic heavy metal that acts as a nonspecific poison, affecting all body systems (Hernández and Margalida 2009). Blood-Pb concentrations obtained in this study were slightly higher than those obtained by García-Fernández et al. (1995) and García-Fernández et al. (1997) in common buzzards from south-eastern Spain. According to Franson (1996), 90.3 % of the common buzzards analyzed in our study had blood-Pb concentrations compatible with an absence of abnormal Pb exposure, 8.6 % had Pb levels indicative of subclinical exposure, and only 1.1 % had a potentially lethal blood-Pb concentration. Taking hepatic-Pb concentrations into consideration, several researchers determined higher concentrations in the same species than those we quantified (Alleva et al. 2006; Battaglia et al. 2005; Jager et al. 1996; Licata et al. 2010; Naccari et al. 2009; Pain et al. 1995; Pérez-López et al. 2008). In contrast, García-Fernández et al. (1995) and García-Fernández et al. (1997) quantified lower hepatic- and renal-Pb concentrations. According to the ranges established by Pain et al. (1995), none of the studied animals exceeded the calculated dry weight threshold for massive exposure and most of them had very low hepatic and renal Pb concentrations of <2 μg/g, with many <1 μg/g d.w., indicating a safe environmental exposure. These results suggest that common buzzards in Portugal are exposed to relatively low levels of Pb. However, some studies provide evidence that low-level Pb exposure, although not causing the clinical symptoms of classical Pb poisoning, may nevertheless have subtle detrimental effects on normal behavior and cognitive function (Burger and Gochfeld 2000; Scheuhammer 1987), while Pb poisoning has been recognized as one of the most significant causes of mortality in raptors (Pain et al. 2005).

There are very few studies on Hg concentrations in birds of prey and, as far as we know, this is the first report on Hg blood concentrations in the common buzzard and, in Portugal, the first in raptors. Tartu et al. (2013) and Goutte et al. (2014) evaluated the effect of Hg in seabirds predators, and they conclude that Hg exposure could affect the ability of modulate their reproductive effort. As threshold-effects levels for Hg have yet to be established for bird blood, it is unclear whether Hg levels were high enough to pose a risk to any of these birds. Considering the hepatic and renal Hg concentrations, other authors have quantified higher Hg concentrations in the common buzzard (Alleva et al. 2006; Castro et al. 2011). Hg concentrations observed in our birds suggested that a source of Hg does exist. According to the previous information and to Scheuhammer (1987), the common buzzards studied are chronically exposed to normal background levels.

It is perhaps because Portugal is a small country (with a maximum extension in length of 561 and 218 Km in width) that we did not observe significant differences between areas of origin in the concentrations of various elements analyzed.

Age was the only factor explaining Cd accumulation in both the liver and kidney, as observed in other studies with wild birds (García-Fernández et al. 1996; Naccari et al. 2009; Ribeiro et al. 2009). With continued exposure, even at low levels, this nonessential element is accumulated throughout the life span of birds, due to its extremely long biological half-life once bound to metallothionein in tissues and its slow elimination from these tissues (Scheuhammer 1987; Furness 1996). Age also had an influence on blood Pb and Hg concentrations, but this influence was not verified in hepatic and renal concentrations. Differences in blood Hg levels between age classes seem to be related with prey-size selection and stage of juvenile feather moult (Evers et al. 2005). Knowing that blood Hg is strongly influenced by dietary uptake, these age-related differences could be due to adults and young eat different foods or eat different proportions of the same foods (Burger and Gochfeld 1997). Apart from the dietary intake, the Hg concentration in blood reflects physiological influences, such as mobilization and storage in feathers and eggs (Dauwe et al. 2000, 2003; Honda et al. 1986). The amount of Hg eliminated into eggs is usually small compared to the amount transferred into feathers during the moult (Furness 1993). Feather moult and growth is the main Hg excretion pathway (Braune and Gaskin 1987; Honda et al. 1985; Monteiro and Furness 2001). The ability to rapidly transfer blood Hg into growing feathers partly accounts for the significant difference in blood Hg levels between adults and juveniles prior to fledging (Evers et al. 2005). Although the Pb excretion into growing feathers occurs to a lesser extent compared with Hg (Dmowski 1999; Furness 1993), the differences between adults and juveniles in blood Pb concentrations could also be related to the stage of juvenile feather moult and growth. Once blood Pb concentrations reflect immediate dietary intake (Furness 1993), the differences for blood Pb concentrations between adults and juveniles could also be explained by the considerations of feeding behavior (Burger and Gochfeld 1997).

Only Pb- and Hg-blood concentrations were influenced by season. Pb poisoning in raptors is likely to be more significant in autumn and winter, since the proportion of carrion taken by certain species may be higher in these seasons. In addition, since waterfowl and other game species are generally hunted during autumn and winter, killed, crippled, and poisoned individuals provide a readily available Pb-contaminated food source (Pain and Amiard-Triquet 1993). Common buzzards often act as scavengers and, in this way, are more likely to be exposed to the lead shot prevalent in small game species (Battaglia et al. 2005). This fact could help to understand the generally higher blood Pb concentration quantified in autumn and winter. In the case of Hg, possible explanations for the significant differences found between the different seasons are migration, diet (Eisler 1987), and moult. It is during the moulting that Hg is incorporated in the keratin structure of the feathers, thus reducing the Hg levels in blood (Braune and Gaskin 1987; Honda et al. 1986; Monteiro and Furness 2001).

Regarding correlations, we found that Pb- and Hg-blood concentrations were statistically related to their corresponding concentrations in the liver or kidney—which suggests that blood concentrations of these metals may be a useful indicator of the degree of recent exposure. García-Fernández et al. (1996) showed that blood-Cd concentration may also be a useful indicator of the degree of exposure to this metal. In this study, due to the large number of samples in which it was not detected, it was not possible to show whether blood could be a useful indicator of the degree of exposure to Cd. Kidney samples could be used to assess chronic exposure to As, Cd, Pb, and Hg, a working hypothesis substantiated by the significant correlations between liver and kidney concentrations of these trace elements.

Conclusion

In general, Hg was the element studied present in the highest concentrations in the three types of samples, and the kidney was the sample with the highest concentrations of each element. If possible, in future studies, it would be important to exclude birds that had migrated from northern Europe and to measure Hg concentrations in feathers, in order to further examine the causes of the higher blood-Hg concentrations in winter and autumn. The generally higher blood-Pb concentrations quantified in autumn and winter are possibly due to birds' higher consumption of individuals crippled and poisoned through the hunting of small game species, which indicates that future measures regarding hunting practices are necessary in order to avoid high Pb exposure and/or Pb poisoning in wild birds. Although raptors 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 this study generally had low levels of heavy metals in blood and tissues, compared with other authors. However, there are unknown sources of exposure to the trace elements studied, so further studies are needed to determine their origin.

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