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ORIGINAL PAPER

ECOLOGICAL FACTORS HELPING TO AVOID THE TOXIC ELEMENT ACCUMULATION IN LIVERS OF LESSER SPOTTED EAGLES (*CLANGA POMARINA* BREHM) FROM EASTERN POLAND

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Abstract

The lesser spotted eagle is one of the most numerous species of eagles of Eurasia given high protection priority. However, it belongs to scarsely studied birds from the above area. The livers used in this study were obtained from moribund individuals delivered to the rehabilitation centres or veterinary doctors close to the raptors' nesting places in Eastern Poland. The aim of the study was to estimate the levels of accumulation of some toxic and essential elements in samples of livers from the lesser spotted eagle and to perform analyses of ecological factors influencing toxic element concentrations. The content of elements in the samples was determined by the method of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The following accumulation pattern for toxic elements was found: Pb > Cd > Hg. In respect of the concentrations of essential elements, the following pattern was observed: Fe > Mg > Zn > Cu > Mn. It was demonstrated that the elevated concentration of cadmium is usually accompanied by high levels of iron and zinc. Simultaneously, a high concentration of hepatic mercury was accompanied by high levels of selenium and iron as well. In contrast to many other species of European birds of prey, the examined livers of lesser spotted eagles showed lead concentrations not indicative of raised exposure to Pb resulting in subclinical toxicity. Concerning Se, the Se concentration exceeded 5.0 mg kg^{-1} in only two liver samples. The absence of game in the eagles' diet during the breeding season and also the autumnal migration are the factors which allow lesser spotted eagles to avoid pollution from lead bullets.

Keywords: lesser spotted eagle, essential elements, heavy metals, liver.

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INTRODUCTION

The lesser spotted eagle (*Clanga pomarina* Brehm) is one of the most numerous species of eagles of Eurasia given high protection priority. However, unfortunately, it belongs to the least studied raptors in this area. The eagle breeds in Central and Eastern Europe and south-eastward to Turkey. In Europe, the breeding population is estimated to be 16.4 - 22.1 thousand breeding couples (Birdlife International 2016). In contrast to the studies on the distribution, breeding and food ecology of this species (Zub et al. 2010, Vali 2012, Maciorowski et al. 2015), no wider research on the toxic and essential elements in organs of these birds has been performed. Sparse reports concerning this species (1-2 examined specimens) from the same area we investigated are provided by Falandysz et al. (1987), Amarowicz et al. (1989), Komosa, Kitowski (2008). For this reason, it seems necessary to obtain data both on the accumulation levels of toxic elements (whose adverse impact is well known) and on the levels of essential elements (which obviously may have toxic effects at elevated concentration levels) in livers of this species (Koivula, Eeva 2010). The liver, like other soft tissues, is useful material for bioindication studies because it reflects the current exposition to various elements, in contrast to bones, which manifest effects of long term exposure (Mateo et al 2003, Fedynich et al 2007).

The aim of the study was to investigate the accumulation levels of some toxic and essential elements in many liver samples from the lesser spotted eagle, and to conduct analyses of the influence of ecological factors on the concentrations of these elements.

MATERIAL AND METHODS

The liver samples (n = 11) of lesser spotted eagles used in this study were obtained from severely wounded and moribund individuals delivered to the rehabilitation centres or veterinary doctors close to the raptors' nesting places in Eastern Poland (Lublin, Białystok and Olsztyn regions) between 2010 and 2012. The total length of stay of these individuals in the clinics or rehabilitation centres did not exceed one week because the eagles died despite continuous veterinary treatment. Following the extraction from the eagles' bodies, the livers were stored in freezers at -30°C. Prior to the measurements, the livers were freeze-dried and ground in a ceramic mortar. All glassware and utensils were rinsed with tap water, soaked in an acid bath (5M HNO_3) for 24 h, rinsed with demineralised water and dried under a laminar flow hood before use, in order to minimise the risk of any metal contamination. Weighed portions (usually 500 ± 1 mg each sample) were poured with 10 mL of concentrated HNO_3 (Sigma Aldrich) and subjected to wet-ashing. Mineralisation was carried out in a Microwave Digestion System with optimal temperature and pressure applied to each individual sample being monitored throughout the acid digestion procedure (Berghof Speedwave) in Teflon vials (DAP 100 type). Mineralisation ran according to the following scheme: 15 min with the temperature rising from room temp. up to 140°C, 5 min. at stable temp. of 140°C, 5 min with the temp. rising from 140°C up to 170°C, 15 min at 170°C and finally cooling down to room temperature (variable time). The pressure over the whole mineralisation process did not exceed 12 bar (1.2 MPa). Clear solution was obtained after the mineralisation process was completed. Next, the solution was cooled down to room temperature, transferred to a 50-mL volumetric flask and replenished with demineralised water (ELGA Pure Lab Classic). In this study, an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) from Thermo Scientific iCAP Series 6500, equipped with a charge injection device (CID) detector, was used for the determination of elements. The spectrometer was controlled by a PC based iTEVA software. The following instrumental parameters were set: RF generator power 1150 W, RF generator frequency of 27.12 MHz, coolant gas flow rate – 16 L min^{-1}, carrier gas flow rate – 0.65 L min^{-1}, auxiliary gas flow rate – 0.4 L min^{-1}, max. integration time – 15 s, pump rate – 50 rpm, viewing configuration – Axial, replicate – 3, flush time – 20 s. The following multi-element stock solutions (Inorganic Ventures) were used as standards:

A) Analityk – 46: ^{63}Cu, ^{57}Fe, ^{24}Mg, ^{31}P, ^{39}K, ^{23}Na in 5% HNO_3 – 1000 mg kg^{-1};

B) Analityk – 47: ^{27}Al, ^{75}As, ^{111}Cd, ^{52}Cr, ^{208}Pb, ^{55}Mn, ^{201}Hg, ^{60}Ni, ^{45}Sc, ^{79}Se, ^{88}Sr, ^{51}V, ^{66}Zn in 10% HNO_3 – 100 mg kg^{-1}.

All concentrations obtained in this study are given in mg kg^{-1} dry weight (dw). For comparison with results expressed in mg kg^{-1} of fresh weight, the factor 4.0 was adopted according to Kalisińska et al. (2009). As it was found in the literature, liver lead concentrations <2.0 mg kg^{-1} dw were considered as the background concentration (Wobeser 1997) and values > 6.0 mg kg^{-1} dw were perceived as a diagnostic level indicative of elevated exposure to lead, resulting in subclinical toxicity (Martin et al. 2008). In the case of cadmium, some authors (Burgat 1990, Battaglia et al. 2005) suggested that Cd levels ≥3.0 mg kg^{-1} dw in the liver might indicate increased environmental exposure.

Statistical analysis

The results were analysed statistically using Statistica 6.1 software. Geometric means and standard deviations were calculated for every element studied. The Sperman range correlations were employed to determine possible correlations among concentrations of the elements.

In order to assess differences between concentrations of the groups of elements tested, the Kruskal-Wallis test was used, and *p*-value of less than 0.05 was considered significant.

RESULTS

Elemental concentrations in livers of the examined lesser spotted eagles are shown in Table 1. Table 2 presents the demonstrated Spearman range

Table 1

Descriptive statistics for selected elements in liver samples (S1-11) of eleven studied individuals of the lesser spotted eagle *Clanga pomarina* (mg kg^{-1} dw)

Specification	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Pb	Se	Sr	V	Zn
S1	2.896	0.496	40.51	4204	1.591	809.4	9.919	2.072	5.350	0.398	0.399	400.1
S 2	0.122	0.309	11.87	1067	0.042	680.5	15.44	0.448	2.768	0.097	0.064	95.31
S 3	0.803	0.288	10.65	1202	0.515	536.2	7.285	0.666	2.216	0.140	0.067	81.28
S 4	1.071	0.282	12.26	997.1	0.450	577.4	9.041	1.393	2.969	0.273	0.048	117.6
S 5	1.261	0.453	18.38	1439	1.839	656.7	9.995	0.916	4.747	0.825	0.185	103.0
S 6	3.425	0.438	15.04	3883	1.757	805.8	14.23	1.656	2.855	0.086	0.187	227.4
S 7	1.426	0.375	16.10	2111	1.614	719.8	14.65	0.973	3.404	0.003	0.047	97.32
S 8	0.894	0.458	13.87	1958	1.948	801.0	13.68	1.012	3.880	0.654	0.052	71.96
S 9	0.477	0.559	13.48	1141	1.029	859.1	11.0	1.005	5.048	1.269	0.113	86.42
S 10	0.387	0.472	11.83	1852	1.922	795.3	9.538	0.697	4.503	0.481	0.118	81.26
S11	0.129	0.300	11.06	1007	0.049	688.5	15.83	0.484	2.687	0.079	0.054	96.23
GM	0.746	0.392	14.68	1660	0.684	713.7	11.17	0.927	3.531	0.187	0.094	113.5
SD	1.079	0.096	8.482	1135	0.764	103.7	2.800	0.501	1.083	0.393	0.106	98.32

GM – geometric mean, SD – standard deviation

Table 2

Statistical correlations between the hepatic concentrations of the tested elements (Spearman's test)

Elements	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Pb	Se	Sr	V	Zn
Cd	-	0.15	0.78***	0.70**	0.44	0.24	-0.1	0.80***	0.33	-0.06	0.29	0.67**
Cr		-	0.50	0.55	0.55	0.83***	-0.02	0.37	0.85***	0.63**	0.55	-0.10
Cu			-	0.66*	0.46	0.45	0.09	0.68**	0.69**	0.20	0.31	0.59
Fe				-	0.68**	0.55	-0.01	0.54	0.43	-0.01	0.46	0.22
Hg					-	0.38	-0.19	0.40	0.56*	0.43	0.26	-0.20
Mg						-	0.25	0.54	0.62**	0.26	0.40	0.06
Mn							-	-0.36	-0.32	-0.29	-0.23	-0.13
Pb								-	0.54	0.23	0.27	0.53
Se									-	0.70**	0.41	0.15
Sr										-	0.17	-0.13
V											-	0.33

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$

correlation coefficients between the hepatic concentrations of the analysed elements. Among the eleven specimens of the lesser spotted eagle, the accumulation of cadmium exceeded 3.0 mg kg^{-1} dw in just one case of a single specimen, while another specimen showed a Cd level close to the limit which might indicate increased environmental exposure. In three other specimens, the Cd concentrations > 1.0 mg kg^{-1} dw were found in the livers (Table 1). As it can be observed, the hepatic concentrations of cadmium correlated strongly with the hepatic concentrations of copper, iron and lead (Table 2). In almost all the examined liver samples except one, the hepatic lead concentrations below the background level were found. Thus, none of the examined specimens showed Pb concentrations to be diagnostic of elevated exposure to Pb resulting in subclinical toxicity (Table 1). On the other hand, it was confirmed that the hepatic concentrations of lead, apart from Cd, were statistically significantly correlated with the hepatic copper concentrations (Table 2). In seven specimens, the accumulation of Hg in the liver exceeded 1.0 mg kg^{-1}. The increasing concentration of mercury was accompanied with increasing concentrations of iron (Table 2). In the examined samples the following sequence of geometric mean concentrations of elements was shown: Pb > Cd > Hg. Unlike a relatively high similarity of geometric means (Table 1), the median values amounting to 0.989 mg kg^{-1}, 1.602 mg kg^{-1} and 0.982 mg kg^{-1}, respectively, differed statistically (Kruskal-Wallis test: $H = 0.352$, $df = 2$, $p = 0.839$). All the examined individuals cumulated >2.0 mg kg^{-1} of selenium. In the case of two specimens hepatic levels of Se > 5.0 mg kg^{-1} were observed. Increasing hepatic concentration of mercury was accompanied by an increase of the concentration level of selenium (Table 2). All the examined individuals accumulated > 10.0 mg kg^{-1} dw of hepatic Cu. The levels of hepatic copper were correlated with the levels of Fe, Pb and Se as well (Table 2). The geometric means of the levels of copper and manganese concentrations were very similar (Table 1). Highly elevated accumulation of iron was detected in two liver samples, where the Fe level was > 3000 mg kg^{-1} dw (Table 1). The individuals with the maximum levels of Fe accumulated also had the highest amounts of Cd. The geometric mean of the accumulated Zn was relatively low among all the specimens of eagles (Table 1). However, in one eagle's liver, the Zn concentration was so high that it exceeded the level responsible for a sub-lethal or poisoning effect (Table 1). Relatively high correlations were observed between the concentrations of cadmium and zinc (Table 2). Considering the geometric means of the hepatic concentrations of essential elements, the following sequence of accumulation was observed: Fe > Mg >Zn > Cu >Mn.

DISCUSSION

Amarowicz et al. (1989), found 0.05 mg kg^{-1} ww (0.20 mg kg^{-1} dw) of cadmium in the liver of a single specimen of the lesser spotted eagle from North

Poland, which was 1.6-times lower than the lowest level found in our study. On the other hand, this result coincides with the data obtained by Kenntner et al. (2007) for golden eagles (*Aquila chrysaetos* L.). Those birds inhabiting the European Alps accumulated the median level of Cd of 0.20 mg kg^{-1} dw (0.050 mg kg^{-1} ww) at the maximum level of 0.265 mg kg^{-1} (1.060 mg kg^{-1} ww) (Kenntner et al. 2007). The concentration found in a sample originating from the Alps was much lower than that observed for the lesser spotted eagles from East Poland. Highly variable levels of hepatic Cd are reported in other birds of prey. For example, Hontelez et al. (1992) found a median hepatic Cd concentration of 0.6 mg $\cdot kg^{-1}$ in common buzzards (*Buteo buteo* L.) living in the Netherlands, which is similar to our results. On the other hand, Jager et al. (1996) reported the median Cd level of 1.20 mg $\cdot kg^{-1}$ (range 0.170-13.45 mg kg^{-1}), which is almost two-fold higher than our determinations. It has been shown that at relatively high levels of Cd in the environment, the cadmium content of key human and animal organs closely reflects Cd levels induced by industrial emissions and fertilizers (Pan et al. 2010, Satarug et al. 2011).

While East Poland is mostly rural, the consumption of fertilizers, for certain economic reasons, is low in most areas of this part of Poland. This observation was supported by Battaglia et al. (2005) who showed that the cadmium levels in the liver might indicate increased environmental exposure only in two out of the eleven individuals examined. It seems that the threat posed by Cd to lesser spotted eagles in the territory of East Poland will increase in the near future due to a documented tendency of birds to colonize small forest patches between large and intensively used fields (often monocultural) and meadows (Wójciak et al. 2005). Such sites guarantee the abundance of prey (amphibians *Amphibia*, voles *Microtus* sp., European hamsters *Cricetus cricetus*, moles, small birds, etc.) of the sizes preferred by eagles (Zub et al. 2010).

Concerning lead absorption, this metal is deposited in various tissues, primarily the liver, kidneys and bones. Whilst lead in the liver and kidneys has a turnover rate (biological half-life) of weeks to months, it is retained in bone for years, thus reflecting both acute and lifetime chronic exposure from all sources (Pain et al. 2005). Most of the tested lesser spotted eagles had very low hepatic Pb concentrations, i.e. below 2 mg kg^{-1} (except one individual) and in just four cases the Pb level exceeded 1 mg kg^{-1}, which implied safe environmental exposure. These results suggest that the lesser spotted eagles in East Poland are exposed to relatively low levels of Pb, and none of the individuals was exposed to lead resulting in subclinical toxicity (Pain et al. 1995, Martin et al. 2008). Contrary to other raptors, there are three main behaviour traits of the lesser spotted eagle which allow this bird to avoid the intoxication with lead. The first one is the opportunistic capture of prey in the form of small mammals and other small and medium-size prey (such as small rodents, moles, small birds, and amphibians). Studies from East Poland have shown that 70% of the analysed prey had body mass below 50 g (Zub et al. 2010). For eagles living over the entire geographical range, the prey is dominated by species which are relatively easy to capture and not very agile. Such preferences allow eagles to avoid excessive accumulation of Pb. Another feature of lesser spotted eagles is their preference for rather dry areas, especially large clear-cut meadows in the vicinity of their nests (MIRSKI 2009), and therefore these birds avoid waterfowl. Reports from wetlands of Poland indicate that the great spotted eagles (*Clanga clanga* Pall.), which definitely prefer wetter areas and frequently feed on waterfowl (MACIOROWSKI et al. 2015), succumb to a higher Pb contamination than do the lesser spotted eagles. Some studies conducted in East Poland showed that the great spotted eagles could have an almost 8.5-times higher Pb concentration in bones than did the lesser spotted eagles (KOMOSA, KITOWSKI 2008). The third characteristic of this species' behaviour is the ability to migrate. These eagles are long-distance migrants (MEYBURG et al. 2004), which usually arrive in European breeding areas in April and fly away to wintering grounds in South Africa at the beginning of September (MEYBURG et al. 2004). Such behaviour allows them to avoid heavy winter time, when wintering birds of prey, especially in East Poland, are exposed to low temperatures and suffer because of the depletion in food resources. AMAROWICZ et al. (1989) reported the level of 1.25 mg kg^{-1} ww (6.0 mg kg^{-1}dw) of hepatic Pb at a single lesser spotted eagle from North Poland, which may indicate that this bird had foraged on bodies of game animals to a greater extent than the individuals from East Poland examined in the present study. KENNTNER et al. (2007) reported the median level of hepatic Pb of 1.64 mg kg^{-1} dw (0.410 mg kg^{-1} ww) for livers of seven golden eagles from the European Alps. As much as 26.67 mg kg^{-1} dw (6.67 mg kg^{-1} ww) of lead was found in a single specimen. Among 372 specimens of the bald eagle (*Haliaeetus leucocephalus* L.) and the golden eagle from Western Canada, 10% were diagnosed as lead poisoned. Golden eagles with high concentrations of Pb tend to be found more frequently during or soon after the autumn hunting season than individuals with background lead concentration (WAYLAND et al. 2003). Contrary to the lesser spotted eagles leaving Europe for the winter season, local buzzards must tackle winter food depletion. Therefore, in areas characterised by harsh winters, birds suffering from food shortages may exploit game animals, including animals wounded and unrecovered by hunters or their carcasses (KENNTNER et al. 2003, VALKAMA et al. 2005). Therefore, such feeding habits can be a serious threat to buzzards from East Poland, where winters are severe due to the climatic impact of the continental zone. Although a relatively low geometric mean of hepatic Pb was found, i.e. 1.98 mg kg^{-1} (range: 1.480-3.050 mg kg^{-1} dw) in the previous experiment (KOMOSA et al. 2012), in other liver samples analysed the concentrations of lead were determined to be above 6 mg kg^{-1} dw and a few samples revealed lead concentrations as high as over 15 mg kg^{-1} dw, which is considered as diagnostic lead poisoning (PAIN et al. 1995, MARTIN et al. 2008). These results suggest a notable scale of the risk associated with eating game animals. Other authors reported very similar hepatic Pb concentrations in buzzards from different parts of Europe, e.g. 0.70 mg kg^{-1} dw (Hontelez et al. 1992) or 1.34 mg kg^{-1} dw (Pain et al. 1995). Relatively higher concentrations of this element were found by Jager et al. (1996): 3.30 mg kg^{-1} dw, while much higher values were reported by Battagalia et al. (2005): 47.70 mg kg^{-1} dw.

The food preferences of the lesser spotted eagle described above also contribute to the prevention of mercury accumulation. Several authors have shown a strong correlation of hepatic Hg accumulation in birds with such feeding preferences as using wetlands as foraging sites as well as eating water animals, especially fish (Clarkson, Magos 2006). For example, Falandysz et al. (2001) found 5.80 mg kg^{-1} of Hg in livers of the white-tailed eagles (*Haliaeetus albicilla* L.). On the other hand, the eagles examined in this study originated from areas with no considerable industrial sources of mercury, and the most of the examined eagle individuals accumulated mercury at the levels similar to that reported by Amarowicz et al. (1989), i.e. 0.282 mg kg^{-1} ww (1.13 mg kg^{-1} dw). Such levels were similar also to those found by Kenntner et al. (2007) among golden eagles from the European Alps, which accumulated mercury on a median level of 0.760 mg kg^{-1} dw (0.190 mg kg^{-1} ww). Reports concerning Hg levels higher than those reported by Kenntner et al. (2007) or the levels shown in lesser spotted eagles herein appear only occasionally. For goshawks (*Accipiter gentilis* L.) from Spain the median Hg hepatic level was 0.236 mg kg^{-1} dw and 0.182 mg kg^{-1} dw, for males and females, respectively (Castro et al. 2011). A German study on the goshawk's livers showed median levels of hepatic mercury of 0.069 mg kg^{-1} ww (about 0.276 mg kg^{-1} dw).

The accumulation of Hg is frequently correlated with the accumulation of Se, as the latter element may be a protective agent against highly neurotoxic Hg (Clarkson, Magos 2006, Scheuhammer et al. 2008). Some papers report significant positive correlations between both elements in livers and blood of eagles (Scheuhammer et al. 2008, Harmata 2011), which are also confirmed in the present study (see Table 2).

CONCLUSIONS

The following accumulation sequence of essential and toxic elements in livers was found: Fe > Mg > Zn > Cu > Mn and Pb > Cd > Hg, respectively.

Contrary to other species of European raptors, no elevated levels of lead were found in the livers of lesser spotted eagles. The examined eagles use dry areas as hunting grounds during breeding, and by migration in autumn they avoid feeding on game during winter, which protects them from being exposed to lead bullets. It was found that elevated concentrations of Cd were accompanied by the high accumulation of iron and zinc. The accumulation of Hg in livers of lesser spotted eagles was correlated with the accumulation of Se and Fe.

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