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Factors affecting trace element accumulation in livers of avian species from East Poland

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Abstract: The aim of this study was to determine the concentration of 12 elements in the livers of 14 different species of birds from East Poland, as well as factors affecting their accumulation. The greatest amount of Cd was found in the livers of woodcocks. Analyses showed that Cd accumulation in the liver was associated with the consumption of soil invertebrates. Our data show that some corvids were the most vulnerable to the effects of lead bullets. In the livers of some of them, lead levels exceeded 6 mg/kg. Expansion of food niches in the direction of omnivorous species limited the accumulation of Hg and Cu, which resulted in low concentrations of these elements in omnivorous species. On the other hand, deep specialization in catching fish favored accumulation of Hg and Cu. Accumulation of Mg and Mn may occur due to foraging in wetlands. Liver concentrations of Se that may result in sublethal effects and reproductive impairments were found in grey herons, cormorants, and mallards.

Key words: Essential elements, heavy metals, birds, liver, contamination

1. Introduction

Birds have been recognized as good spatiotemporal integrators of pollutions because of their wide distribution, feeding at different trophic levels, long lifespan, and abundance (Jerez et al., 2013; Kalisinska et al., 2013; Mansouri and Majnoni, 2014). Birds are susceptible to bioaccumulation of pollutants mainly through the consumption of contaminated food. These species can provide interesting data to monitor the quality of the environment (Nowrouzi et al., 2012; Kim and Oh, 2013; Mansouri and Majnoni, 2014). They are often used for the indication of heavy elements because, in nature, these elements are persistent and easily accumulate in their key organs, such as the liver or kidneys. Some elements can biomagnify up the food chain and accumulate with age (Evers et al., 2005; Kalisinska et al., 2010; Nowrouzi et al., 2012). Their concentrations are generally greater in birds at higher trophic levels. Some works using birds as bioindicators were conducted in areas subjected to different forms of anthropopression (Taggart et al., 2006; Lucia et al., 2010). In contrast, research on species (including birds) commonly occurring in areas with moderate human impact where spot pollution of biotas can occur are conducted less frequently. Furthermore, few works exist on the interactions between the elements already accumulated in the key organs (Wayland et al., 2001; Kim and Oh, 2012; Kim and Oh, 2013).

Concerning areas of East Europe, including Poland, there are only scarce reports dealing with factors affecting the accumulation of trace elements in the livers of avian species. If found, existing studies generally relate to the concentration of elements and rarely consider interactions among toxic heavy metals and essential elements (Pilarczyk et al., 2012; Binkowski et al., 2013; Kalisińska et al., 2013). The objectives of this study were to use birds as a sentinel species to determine if birds in East Poland are potentially acquiring unhealthy burdens of elemental contaminants, to describe hepatic concentrations of elemental contaminants in birds collected in East Poland, and to determine and discuss interactions among toxic heavy metals and essential elements in the livers of one of the examined species.

2. Materials and methods

2.1. Studied species and their origin

Experimental material consisted of the livers (collected from 80 specimens) of the following birds from 14 species: raven *Corvus corax* (2), rook *Corvus frugilegus* (24),

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hooded crow *Corvus cornix* (6), jackdaw *Corvus monedula* (2), magpie *Pica pica* (2), Eurasian jay *Garrulus glandarius* (10), Eurasian collared dove *Streptopelia decaocto* (2), mallard *Anas platyrhynchos* (8), woodcock *Scolopax rusticola* (6), grey heron *Ardea cinerea* (3), great cormorant *Phalacrocorax carbo* (2), European herring gulls *Larus argentatus* (3), common gull *Larus canus* (5), and black-headed gull *Chroicocephalus ridibundus* (5).

All studied birds originated from different habitats in East Poland (Warsaw, Białystok, Lublin, and Rzeszow). Woodcocks were migratory birds moving through eastern Poland, which during migration collided with high buildings and other infrastructure and were found dead. Mallards were collected from an urban population nesting in Warsaw (capital of Poland - 2 million inhabitants) and were victims of collisions with cars or getting lost indoors.

2.2. Analytical procedures

The livers used in this study were obtained from wounded individuals delivered to rehabilitation centers or veterinary clinics close to the site of death between 2011 and 2014 in East Poland. The birds died despite persistent veterinary treatment, or they were untreatable upon delivery and were administered a lethal injection to spare them suffering. Their total stay in the clinics or rehabilitation centers did not exceed 1 week. Following extraction from the birds' bodies, the livers were stored in freezers at –30 °C. Prior to the measurements, the livers were lyophilized and ground in a ceramic mortar. All glassware and utensils were rinsed with tap water, soaked in an acid bath (5 M HNO_3) for 24 h, rinsed with demineralized water, and dried under a laminar flow hood before use in order to minimize the risk of any metal contamination. Weighed portions of the sample (500 ± 1 mg) were poured with 10 mL of concentrated HNO_3 (Sigma Aldrich, St. Louis, MO, USA) and subjected to wet-ashing. Mineralization was carried out using the Microwave Digestion System with optical temperature and pressure monitoring of each individual sample during acid digestion (Speedwave; Berghof Group, Eningen, Germany) in Teflon vials (DAP 100 type), according to the following scheme: 15 min from room temperature to 140 °C, 5 min at 140 °C, 5 min from 140 °C to 170 °C, 15 min at 170 °C, and finally cooling to room temperature (variable time). The pressure over the whole process did not exceed a value of 12 bar. After mineralization, a clear solution of elements was obtained. Next, the solution was cooled to room temperature and transferred to a 50-mL volumetric flask, which was filled with demineralized water (ELGA Purelab Classic; Veolia Water Solutions & Technologies, Saint Maurice, France) to the indicated level. An inductively coupled plasma optical emission spectrometer (ICP-OES, iCAP Series 6500; Thermo Scientific, Waltham, MA, USA), equipped with a charge injection device detector, was used for the determination of elements. The spectrometer was controlled by PC-based iTEVA software. The following instrumental parameters were set: RF generator power: 1150 W, RF generator frequency: 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, replicates: 3, flush time: 20 s. The following multielement stock solutions (Inorganic Ventures, Christiansburg, VA, USA) 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}.

In order to validate the analytical method, analyses were run on all liver samples and additionally on a blank (control) sample. Detection limits were established by measuring a blank solution (5% HNO_3). The solution was analyzed seven times, with each analysis having three replicates, and the mean of 3 times the standard deviation value from all of the runs was calculated. To control the accuracy of the method under existing working conditions, a certified reference material, TraceCERT - Periodic Table Mix 1 for ICP (Fluka Analytical, Sigma Aldrich) was used. To calculate the recovery percentage, three randomly selected samples were supplied with known amounts of the analytical standard. The mean percentage recoveries of the analyzed elements were calculated based on the following equation: recovery [%] = (CE / CS × 100), where CE was the experimental concentration determined from the calibration curve and CS was the spiked concentration (Table 1).

2.3. Data analysis

All the concentrations obtained in this study are given in mg/kg dry weight (dw). Hepatic Pb concentrations of ≥6 mg/kg dry mass were considered diagnostic of elevated exposure to lead, resulting in subclinical toxicity. If other authors did not put data in mg/kg dw, conversion from wet weight (mg/kg ww) into dry mass (mg/kg dw) was done based on criteria given by Kalisinska et al. (2004, 2006). A factor of 4.0 was used for liver tissue. Liver lead concentrations of ≥15 mg/kg dw were considered diagnostic of Pb poisoning (Martin et al., 2008). In the case of Cd, Burgat (1990) and Battaglia et al. (2005) suggested that Cd levels ≥3 mg/kg dw in the liver might indicate increased environmental exposure and a concentration of >5 µg/g is a reasonable minimum value associated with significant alteration in metabolism (Fedynich et al., 2007). Liver Hg concentrations of 49–125 mg/kg have been reported for free-living birds found dead or dying (Thompson, 1996). Gasaway and Buss (1972) and Sileo et al. (2003) reported that concentrations of >339 mg/kg

Table 1. Validation of the analytical method: detection limit and recoveries for the studied elements; r – Pearson correlation between element detection and concentration, LOD – limit of detection.

Element	r	LOD [mg L^{-1}]	Recovery [%]
Al	0.9998	0.021	104
As	0.9997	0.015	95
Ca	0.9986	0.002	107
Cd	0.9999	0.001	98
Cr	0.9998	0.002	97
Cu	0.9999	0.002	103
Fe	0.9999	0.012	111
Hg	0.9997	0.052	93
Mg	0.9982	0.005	109
Mn	0.9998	0.002	96
Mo	0.9998	0.022	98
Ni	0.9999	0.001	92
Pb	0.9999	0.008	97
Sc	0.9998	0.002	99
Se	0.9999	0.011	94
Sr	0.9998	0.003	97
V	0.9999	0.003	95
Zn	0.9998	0.010	103

dw (84.8 mg/kg ww) and >280 mg/kg dw, respectively, may be reasonable for sublethal effects in adults. In the case of selenium, concentrations of >12 mg/kg dw (3.00 mg/kg ww) were considered relevant to reproductive impairments, while concentrations higher than 40 mg/kg dw (10.00 mg/kg ww) produced sublethal effects (Pillatzki et al., 2011).

The normality of variable distribution was checked using the Shapiro–Wilk test of normality. Redundancy analysis (RDA) was used to establish the relations between heavy metal concentrations in the context of trophic and habitat preferences of birds. Data necessary for ranking the food and habitat preferences of the examined birds for RDA were taken from the work of Gromadzki (2004), Jerzak et al. (2005), Carpene et al. (2006), and Misztal-Szkudlinska et al. (2011). For 24 rook livers, individual analyses of interactions with other toxic elements were done. In the analysis, a multidimensional relationship between the independent variables (concentrations of elements Cu, Mg, Mn, Se, Sr, V, and Zn) and the dependent variables (concentrations of heavy metals Hg, Cd, Cr, Pb, and Ni) was determined (Table 2). In this case, the greatest explanation capacity among the predictors was shown by vanadium, then zinc, strontium, magnesium, and finally

Table 2. Relationships between the explained variables and the explanatory values. Selected results are statistically significant for P < 0.05. Wilks lambda values range from 0 to 1 (1 – no relationship of predictors with explained variables, 0 – perfect relationship).

Parameters	Cu	Mg	Mn	Se	Sr	V	Zn
Wilks lambda	0.328342	0.313228	0.710647	0.558370	0.286713	0.233982	0.269553
P	0.007050	0.005332	0.426009	0.136881	0.003143	0.000916	0.002167

copper. Although in the case of manganese and selenium there was no association with explained variables, relatively high values for the statistics of the other elements indicated the possibility of creating a model describing the interaction between variables.

In the process of building models, the general regression model (GRM) in Statistica 10.0 was used. Models were built based on the method of least squares. Finally, five models were created to describe the relationship between the elements Hg, Cd, Cr, Pb, and Ni and independent variables (Table 3).

F-test values and the corresponding probabilities indicate that the variances of the variation between groups and within them differ significantly from 1, excluding Cd and Ni. Values of adjusted R and R^2 show that the regression models are very well fitted to the data in the case of Cr, Hg, and Pb, while for Cd and Ni, description of their variability using the dependent variables is not satisfactory (adjusted R^2 at the level of 0.036 for Cd means that only 3.6% of Cd variability is explained by the explanatory variables as other elements; for Ni this value is close to 5%). In light of these results, Ni and Cd were not subjected to further analysis. Analysis of plots presenting predicted versus observed values showed that the predicted values were arranged along a straight line, which acknowledges a good predictive model (Figure 1a). Residuals analysis showed that the residuals have normal distribution (Figure 1b). The analysis also showed that among the residuals neither autocorrelation nor outstanding observations were found.

Table 4 shows the values of beta coefficients (β) and their standard errors (σβ) for models designed for each dependent variable. β coefficients were normalized by the parameters obtained from the regression equation calculated according to Eq. (1):

$$\frac{y_i-\overline{y}}{\delta_y}=\beta\frac{x_i-\overline{x}}{\delta_x}+e, \qquad (1)$$

where:
y_i = independent variable, $\bar{y}$ = the average value of the independent variable, β = beta coefficients, x_i = dependent variable, $\bar{x}$ = the average value of the dependent variable, and *e* = free term.

On the basis of the values of β coefficients, it is possible to evaluate the relative contribution of the independent variables in the description of the dependent variable.

3. Results

Results of laboratory measurements are given in Tables 5 and 6. The highest level of cadmium accumulation in the livers was found in 4 species: woodcock, mallard, black-headed gull, and rook (Table 5). Among these specimens, Cd accumulation levels exceeded 3.0 mg/kg dw in all tested livers (Table 5), and in 5, 6, and 2 specimens of mallard, rook, and jay, respectively. Two rook specimens with Cd levels of 9.24 and 9.86 mg/kg dw were remarkable. RDA (Figure 2; Table 7) showed that increased accumulation of hepatic Cd is correlated with consumption of soil invertebrates.

GRM analysis for rook livers showed no significant impact of any of the tested essential elements on the concentration of Cd (Table 2). Corvids accumulated the highest amounts of hepatic Pb. In the case of rook, hooded crow, magpie, and common gull, a single individual hepatic Pb concentration exceeding 6 mg/kg dw was found (Table 5). The hooded crows with hepatic Pb concentrations of 5.83 mg/kg dw and 21.77 mg/kg dw, respectively, are noteworthy. GRM analysis showed that for rooks, concentrations of lead strongly correlated with the concentrations of Se, Mg, Zn, and V (Table 3). Although the highest mean concentrations of Cr were found in the livers of grey heron and hooded crow (Table 5), the maximum level of this element was detected in a single magpie. In the case of rook livers, GRM analysis showed that Cr concentration is significantly influenced by concentrations

Table 3. Square sum test results. Values in bold are significant at P < 0.05.

	R	R^2	Adjusted R^2	SS - model	MS - model	SS - residual	MS - residual	F	P
Cd	0.536	0.288	0.036	42.18	6.03	104.426	6.143	0.98	0.48
Cr	**0.993**	**0.986**	**0.980**	**3.38**	**0.48**	**0.048**	**0.003**	**170.87**	**0.00**
Hg	**0.899**	**0.810**	**0.731**	**0.19**	**0.03**	**0.045**	**0.003**	**10.33**	**0.00**
Pb	**0.917**	**0.840**	**0.774**	**88.42**	**12.63**	**16.84**	**0.991**	**12.75**	**0.00**
Ni	0.551	0.304	0.058	0.07	0.01	0.165	0.009	1.06	0.43

R – correlation coefficient, R^2 – coefficient of determination (regression), adjusted R^2 – adjusted coefficient of determination, SS – squared deviations from the mean calculated for regression model/for residuals, MS – squared mean deviations from the mean calculated for regression model/for residuals, F – regression statistics determining the linearity of the model and calculated as a relation of MS model/MS residuals, P – statistical significance level.

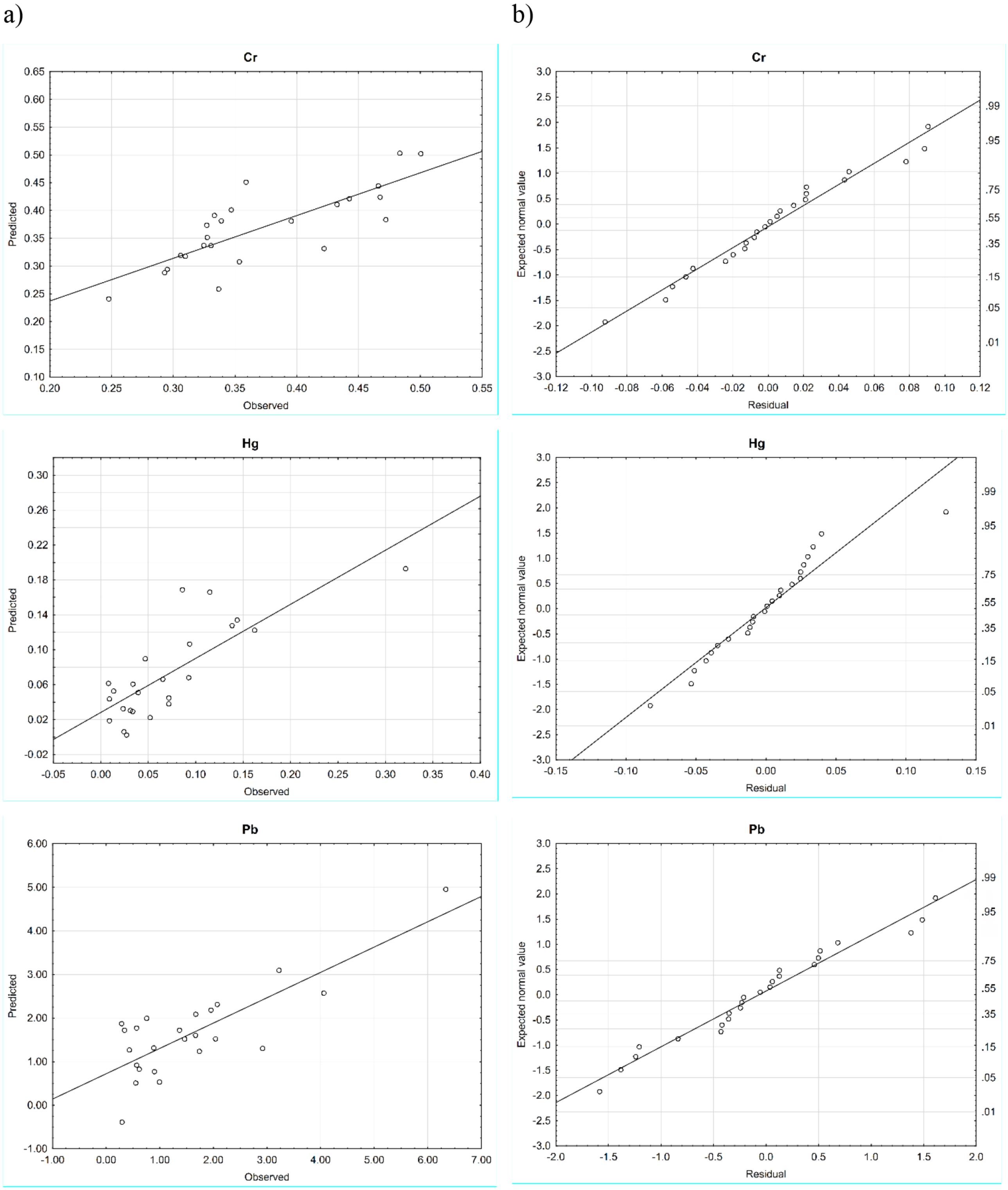

Figure 1. Predicted versus observed values (a) and residuals (b) obtained as results of GRM analysis for Cr, Hg, and Pb.

Table 4. Estimation of β parameters of the constructed models. Values in bold are significant at $P < 0.05$.

Elements	Cr		Hg		Pb	
	β	**σ_β**	**β**	**σ_β**	**β**	**σ_β**
Cu	**0.428356**	**0.151243**	−1.07520	0.557399	0.25306	0.511047
Mg	−0.274521	0.318695	**2.80933**	**1.174538**	**−2.37108**	**1.076867**
Mn	0.126063	0.114026	**−0.97107**	**0.420240**	−0.10566	0.385294
Se	0.121184	0.162406	0.73551	0.598541	1.18526	0.548768
Sr	**0.263201**	**0.074980**	−0.56983	0.276337	0.24672	0.253357
V	0.090792	0.061995	−0.44559	0.228480	**0.95491**	**0.209481**
Zn	**0.318053**	**0.092297**	0.11271	0.340156	**0.88145**	**0.311870**

β – standardized regression coefficient, σ_β – standard errors of β.

Table 5. Descriptive statistics for hepatic concentrations of trace elements [mg/kg dry weight] in livers of 14 species of birds from East Poland. Geometric mean ± SDG (standard deviation of geometric mean) and the range of measurements (below) are given. In the case of limited data, geometric mean and SDG are not given. * – arithmetic mean, Nd – not detected.

Species	N	Cd	Cr	Hg	Ni	Pb	Cu
		GM ± SDG	GM ± SDG	GM ± SDG	GM ± SDG	GM ± SDG	GM ± SDG
C. corax	2	0.33*	0.57*	0.19*	0.26*	0.94*	19.31*
		0.22–0.44	0.30–0.84	0.03–0.36	0.26–0.26	0.91–0.96	17.91–20.71
C. frugilegus	24	1.39 ± 2.52	0.36 ± 0.07	0.06 ± 0.07	0.15 ± 0.10	1.12 ± 1.42	13.32 ± 4.20
		0.07–9.86	0.25–0.50	Nd–0.32	0.04–0. 32	0.29–6.33	8.25–24.20
C. cornix	6	0.62 ± 0.17	0.45 ± 0.15	0.17 ± 0.12	0.09 ± 0.13	3.14 ± 7.35	16.61 ± 5.93
		0.45–0.97	0.31–0.75	0.03–0.39	0.04–0.31	0.56–21.77	8.95–23.93
C. monedula	2	0.74*	0.30*	0.02*	0.19*	2.25*	14.26*
		0.39–1.41	0.28–0.32	0.019–0.03	0.11–0.31	1.21–4.20	12.53–16.22
P. pica	2	0.70*	0.61*	0.14*	0.27*	2.17*	23.42*
		0.47–1.04	0.40–0.94	0.13–0.16	0.27–0.28	0.55–8.62	22.25–24.65
S. decaocto	2	0.61*	0.39*	-	0.04*	0.77*	24.95*
		1.22–0.31	0.391–0.393	Nd–0.01	0.03–0.05	0.80–0.74	15.76–39.50
A. platyrhynchos	8	2.03 ± 2.67	0.39 ± 0.05	0.36 ± 2.71	0.02 ± 0.02	1.79 ± 0.96	39.49 ± 26.59
		0.12–7.18	0.32–0.45	0.26–7.95	Nd–0.06	0.60–3.62	21.56–85.48
G. glandarius	10	1.03 ± 1.39	0.45 ± 0.33	0.12 ± 0.22	0.17 ± 0.13	0.91 ± 1.18	24.77 ± 12.46
		0.03–3.85	0.30–1.39	0.01–0.68	0.02–0.42	0.27–4.09	8.49–47.94
S. rusticola	6	6.39 ± 2.04	0.32± 0.08	0.57 ± 0.35	0.04 ± 0.03	0.68 ± 0.61	21.84 ± 12.26
		3.88–9.46	0.25–0.45	0.22–1.21	0.02–0.10	0.23–1.67	11.70–40.89
A. cinerea	3	0.30*	0.46*	44.61*	0.01*	0.70*	44.61*
		0.27–0.35	0.36–0.58	7.53–18.14	Nd–0.02	0.24–0.96	25.32–69.30
P. carbo	2	0.60*	0.33*	32.20*	0.02*	0.19*	61.49*
		0.38–0.95	0.27–0.40	22.96–45.18	0.01–0.04	0.13–0.27	49.82–75.88
L. argentatus	3	1.00*	0.403*	0.582*	0.286*	1.067*	16.61*
		0.57–1.35	0.35–0.43	0.08–1.42	0.28–0.31	0.62–1.82	13.05–22.88
L. canus	5	0.64 ± 0.34	0.36 ± 0.10	0.19 ± 0.43	0.27 ± 0.02	0.77 ± 2.65	16.25 ± 4.36
		0.39–1.06	0.28-0.53	0.02-0.99	0.25–0.30	0.21–6.41	14.05–24.34
C. ridibundus	5	1.57 ± 0.77	0.32 ± 0.04	0.29 ± 0.74	0.28 ± 0.15	1.43 ± 0.86	16.52 ± 3.05
		1.19–2.67	0.25–0.35	0.02–1.97	0.24–0.37	0.38–2.80	13.40–22.10

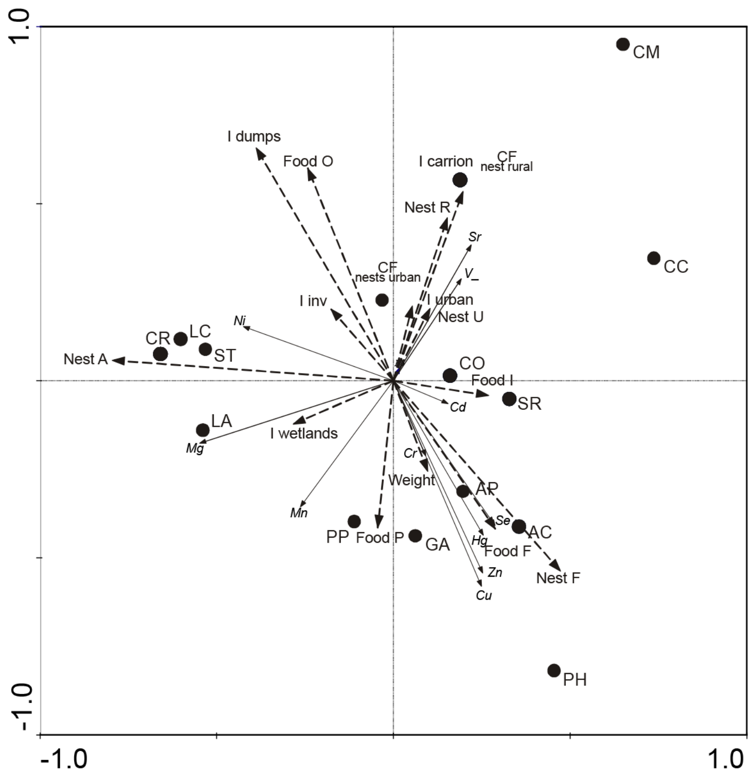

Figure 2. RDA results showing the effect of different ecological parameters (the dotted lines) on the concentration of heavy metals in the livers of studied bird species. Monte Carlo permutation test of significance of all canonical axes: P = 0.002. Eigenvalues: axis 1 – 0.197; axis 2 – 0.138. Abbreviations and scales used for analysis: Species: CC - *Corvus corax*, CF - *Corvus frugilegus*, CO - *Corvus cornix*, CM - *Corvus monedula*, PP - *Pica pica*, SR - *Streptopelia decaocto*, AC - *Anas platyrhynchos*, GA - *Garrulus glandarius*, SR - *Scolopax rusticola*, AC - *Ardea cinerea*, PH - *Phalacrocorax carbo*, LA - *Larus argentatus*, LC - *Larus canus*, CR - *Chroicocephalus ridibundus*. Food preferences: food F – fish, food I – invertebrates, food O – omnivorous, food P – plants. Foraging area: importance of wetlands for foraging (I wetlands), importance of dumps for foraging (I dumps), importance of urban habitats for foraging (I urban): 0 – none, 1 – small, 2 – medium, 3 – big. Nesting site habitat: nest F – forest, nest U – urban habitat, nest A – aquatic habitat, nest R – rural habitats. Vertebrate carrion importance in food (I carrion): 1 – small, 2 – medium, 3 – big; invertebrate importance in food (I inv): 0 – none, 1 – small, 2 – big. Weight – weight of individuals according to Busse (1990).

of Sr, Zn, and Cu (Table 3). RDA analysis showed relations between fish consumption and accumulation of Hg, Se, Cu, and Zn in the livers. The highest hepatic Hg levels were found in cormorants and grey herons. However, only one cormorant (45.18 mg/kg dw of Hg, Table 5) was found with an Hg level close to the lower limit of concentration for free-living birds found dead or dying (see Section 2). RDA showed that, on one hand, fish consumption was associated with maximum Hg accumulation in the liver in two typically piscivorous species (Figure 2). On the other hand, extension of the food niches and withdrawal from deep specialization limited the accumulation of this element. Such a behavior resulted in relatively low hepatic Hg concentrations in omnivorous species such as rook and jackdaw (Figure 2). GRM analysis showed significant Hg interactions with Mg and Mn in rook livers (Table 3). Similarly to Hg, RDA proved the existence of relations between the levels of Cu accumulation and feeding on fish. However, the omnivorous nature of food preferences (wide food niche) of birds decreased their tendency to accumulate Cu (Figure 2). Such an effect is also clearly seen in the corvids examined in this study.

The highest mean hepatic concentrations of Cu were found in piscivorous grey herons. On the other hand, the maximum Cu level was found in a single mallard (85.48 mg/kg dw) and was accompanied by a high (5.61 mg/kg dw) level of Cd. Gulls examined in this study accumulated a mean Cu level of approximately 16.00 mg/kg dw (Table 5) despite different trophic and habitat preferences.

Among 10 of the examined birds, hepatic Zn exceeded 280 mg/kg dw, which may be responsible for sublethal effects in adults. Such a high Zn level was also found in single magpie, herring gull, rook, grey heron, and great cormorant individuals. These values are given in Table 6 as the maxima. RDA showed that Zn correlated with fish consumption, which was similar to Cu, Hg, and Se (Figure

Table 6. Descriptive statistics for hepatic concentrations of trace elements [mg/kg dry weight] in livers of 14 species of birds from East Poland. Abbreviations are the same as in Table 5.

Species	N	Mg	Mn	Se	Sr	Zn	V
		GM ± SDG	GM ± SDG	GM ± SDG	GM ± SDG	GM ± SDG	GM ± SDG
C. corax	2	484.53*	5.95*	3.01*	0.19*	152.04*	0.32*
		460.17–508.90	5.14–6.75	2.57–3.46	0.14–0.24	82.61–221.47	0.28–0.37
C. frugilegus	24	612.77 ± 114.3	6.71 ± 2.16	2.41 ± 0.73	0. 27 ± 0.18	113.32 ± 55.21	0.41 ± 0.30
		422.00–861.50	4.76–13.02	1.48–4.64	0.07–0.82	58.93–303.00	0.12–1.49
C. cornix	6	612.79 ± 56.27	6.31 ± 0.72	3.19 ± 0.80	0.38 ± 0.21	158.04 ± 72.42	0.58 ± 0.19
		525.75–673.27	5.52–6.75	2.34–3.87	0.22–0.47	64.42–238.58	0.41–0.92
C. monedula	2	479.91*	8.44*	2.55*	1.00*	75.16*	0.26*
		409.57–562.33	6.51–10.93	2.20–2.95	0.49–2.05	64.00–88.27	0.21–0.33
P. pica	2	688.09*	6.66*	3.92*	0.25*	206.78*	0.36*
		681.35–694.90	4.75–9.33	3.83–4.00	0.18–0.34	150.77–283.60	0.29–0.44
S. decaocto	2	712.24*	10.13*	1.48*	0.16*	95.18*	0.14*
		677.28–749.00	8.81–11.64	1.65–1.32	0.11–0.24	64.26–140.98	0.12–0.16
A. platyrhynchos	8	629.96 ± 99.83	16.46 ± 2.45	5.46 ± 4.13	0.29 ± 0.15	194.02 ± 86.97	0.20 ± 0.25
		493.55–736.28	14.77–18.80	2.32–14.98	0.18–0.56	61.02–360.63	0.13–0.86
G. glandarius	10	652.84 ± 94.81	6.90 ± 1.82	2.84 ± 0.90	0.21±0.11	214.89 ± 96.46	0.09 ± 0.09
		449.57–800.38	4.42–9.34	2.11–5.10	0.10-0.40	114.2–406.70	0.02–0.35
S. rusticola	6	570.96 ± 161.97	8.82 ± 3.75	2.85 ± 1.36	0. 35 ± 0.20	177.78 ± 75.90	0.15 ± 0.01
		401.20–781.12	4.91–15.11	2.04–5.48	0.12–0.70	87.62–272.68	0.14–0.16
A. cinerea	3	612.48*	15.21*	15.95*	0.14*	252.51*	0.15*
		578.50–643.35	11.13–19.93	5.91–32.31	0.08–0.24	102.57–460.40	0.09–0.19
P. carbo	2	597.83*	16.35*	14.42*	0.16*	276.26*	0.25*
		561.42–636.60	13.74–19.46	12.62–16.47	0.15–0.18	217.92–350.22	0.16–0.41
L. argentatus	3	740.57*	13.06*	1.99*	0.327*	156.78*	0.272*
		715.43–779.05	10.48–16.25	1.64–2.43	0.30–0.35	63.87–308.85	0.07–0.66
L. canus	5	749.02 ± 81.50	13.74 ± 2.00	2.31 ± 0.78	0.22 ± 0.05	100.98 ± 71.01	0.08 ± 0.08
		661.77–879.53	11.91–15.97	1.87–3.77	0.15–0.27	68.87–237.20	0.03–0.22
C. ridibundus	5	736.88 ± 53.74	14.65 ± 2.50	2.61 ± 2.08	0.28 ± 0.15	97.77 ± 27.63	0.05 ± 0.05
		666.07–790.67	11.66–17.70	1.46–7.16	0.11–0.49	76.48–150.18	0.03–0.17

Table 7. Results of forward selection of ecological parameters. Only variables with $P < 0.05$ were included.

Variables	Lambda-A	F	P
Nests in aquatic habitats	0.13	11.64	0.002
Importance of dumps for foraging	0.07	6.61	0.002
Vertebrate carrion importance in food	0.04	4.46	0.010

2). Accumulation of Mg and Mn in livers was supported by foraging in wetlands and limited by foraging in dry areas (Figure 2). On the other hand, accumulation of Mg and Mn in livers of the examined birds limited the accumulation of V and Sr. The highest mean hepatic Mn concentration was found in 3 species of gulls, which accumulated >700.0 mg/kg dw of Mn (Table 6). Grey heron livers also contained the maximum level of selenium, 32.31 mg/kg dw (Table 6), which can result in sublethal effects in adults. Concentrations of Se of >10 mg/kg dw, which may cause reproductive impairments, were also detected in two cormorant specimens and one mallard (Table 6). RDA indicated a relationship between vanadium and strontium accumulation and eating carcasses. Therefore, the largest amounts of these elements were found in the livers of terrestrial birds. On the other hand, foraging in wetlands did not favor their accumulation (Figure 2). Noticeable amounts of V were demonstrated in a single hooded crow and in rooks, and Sr in the hooded crow.

4. Discussion

Woodcocks were characterized by the highest cadmium concentrations in the livers out of all the species. The examined woodcocks surpassed a Cd level that would indicate increased environmental exposure to this element (see Section 2). Analyses indicated a relationship between Cd hepatic concentrations in woodcocks and the consumption of soil invertebrates. Carpene et al. (2006) also showed that Cd accumulation in woodcock kidneys was linked to diet. They observed Cd levels of 15.7 µg/g ww (60.28 mg/kg dw) in kidneys and 2.7 mg/kg ww (10.8 mg/kg dw) in livers, which was 1.7 times higher than the level found in this study. All woodcock livers examined in this study showed Cd levels of >3 mg/kg dw, indicating that these individuals were subjected to increased environmental exposure. In contrast, only 50.0% of the 12 woodcocks studied in Korea exceeded this threshold (Kim and Oh, 2013). For all the woodcocks, the geometric mean of Cd was 3.74 mg/kg dw. Therefore, we suggest that the elevated Cd and Pb concentrations in Eurasian woodcocks are due to contamination in their breeding and/or wintering and migration route sites outside Poland.

Relatively low Cd levels were found in piscivorous species. Horai et al. (2007) noted mean hepatic Cd concentrations of 0.19 mg/kg dw and 0.281 mg/kg dw for grey herons and great cormorants, respectively, which were lower than the concentrations obtained in this study. For grey herons, Hontelez et al. (1992) reported a level similar to the present results (0.4 mg/kg dw Cd). Few reports on cadmium in ducks exist. Fedynich et al. (2007) showed that only 6.5% of 46 adult blue-winged teals *Anas discors* collected in the spring had cadmium concentrations of >5.0 mg/kg dw, which is considered a minimum value associated with significant alteration in metabolism; 37.5% of the mallards examined within this study exhibited such concentrations. Kim and Oh (2012) reported a Cd level of >3.0 mg/g dw (3.48 mg/g dw) for only one Korean mallard. The same authors reported no case from Eurasian wigeons *Anas penelope* (n = 16) and spot-billed ducks *Anas poecilorhyncha* (n = 19). They found geometric means of hepatic Cd for the above species of 0.65 mg/kg dw, 0.37 mg/kg dw, and 0.48 mg/kg dw, respectively. For mallards from Iran, Mansouri and Majnoni (2014) determined a mean hepatic Cd concentration of 1.81 mg/kg dw. Such data correspond well with that for mallards from Warsaw.

GRM analysis of rook livers indicated no influence of Cd on the concentrations of essential elements, which corresponds with RDA results. Soil invertebrates such as earthworms are important components of rook diets, but they have a very wide food spectrum (such as plant food), which protects them from excessive accumulation of Cd. Our studies on rook livers revealed statistically significant interactions between concentrations of lead and zinc. This is due to the tendency of Pb to replace Zn in heme enzymes (Goyer, 1997) and also due to the fact that Pb, acting antagonistically or competing with Zn, may also interfere with the function of cells and weaken their antioxidant defense (Peraza et al., 1998; Hsu and Guo, 2002).

The mean concentrations of Pb and the ranges of the concentrations obtained in this study did not exceed 6.0 mg/kg dw. For certain individuals of corvids and common gulls, concentrations close to or exceeding the above indicated level were found. This may be related to the use of lead bullets by Polish hunters. Hooded crows, apart from ravens, obtain food by consuming carrion. The case of a lead concentration of 21.77 mg/kg dw found in a single hooded crow individual must be considered as diagnostic of Pb poisoning (Martin et al., 2008). Other corvids exploit carrion to a lesser degree; however, in areas with strong hunting pressure, bullets can be confused with stones essential for digesting food. This can explain relatively high hepatic Pb concentrations in some of the rook and magpie individuals. Hepatic concentrations of lead for mallards obtained in this study were relatively low; none of the samples exceeded threshold concentrations of 5 µg/g dw. Kim and Oh (2012) showed that the abovementioned Pb threshold was exceeded by 15.0% in mallards. In Spain, high geometric means of hepatic lead levels reflecting strong hunting pressure were found in mallards: 20.3 mg/kg dw (Mateo et al., 1998) and 17.0 mg/kg dw (Mateo and Guitart, 2003). In this study, geometric means of lead concentrations found in livers of gulls were low: 1.43 mg/kg dw (Table 5). Other authors showed higher hepatic concentrations: 5.1 mg/kg dw in Siberian gull *Larus heuglini* (Hoshyari et al., 2012) and 3.71 mg/kg dw and 4.52 mg/kg dw in black-tailed gull *Larus crassirostris* nestlings from two places (Kim and Oh, 2015).

Low hepatic concentrations of Pb in mallards in the present study may result from the fact that the examined birds originated from Warsaw. Urban mallards may leave the cities and come back (Figley and Van Druff, 1982), but they are much less likely to consume bullets than individuals in areas with high hunting activity.

RDA showed that fish consumption, accompanied by a relatively high body mass, favored the hepatic accumulation of Cu, Zn, and Cr. Despite important biochemical functions on one hand and toxicity on the other, Cr is rarely determined in the livers of birds (Horai et al., 2007). The importance of the liver is not attributed to the accumulation of Cr in birds (Jerez et al., 2013). Experiments seem to indicate the role of the lungs in accumulation of this element, due to registering Cr concentrations 1.5–2.0 times higher than that in the liver (Horai et al., 2007). The maximum Cr concentrations were reported in the lungs of piscivorous birds, although the ability of this element to accumulate in the kidneys is frequently mentioned (Nam et al., 2005a, 2005b; Horai et al., 2007). For some studied species, hepatic concentrations of Cr comparable to those in this study were found. Horai et al. (2007) provided two sets of data for grey heron livers from Japan with 0.386 mg/kg dw and 0.383 mg/kg dw of Cr, respectively, and for great cormorants 0.319 mg/kg dw. Earlier reports concerning the Western reef heron indicate a mean Cr concentration of 1.05 µg/g dw (Mansouri et al., 2012). A slightly higher concentration of Cr was found in the livers of mute swan *Cygnus color* from the Lower Great Lakes (geometric means range: 1.30–1.65 mg/kg dw) (Schummer et al., 2011).

Herring gulls examined in this study accumulated amounts of hepatic mercury similar to the reported levels for mature individuals from the Baltic Sea (0.58 mg/kg dw), but much less than in immature birds (1.05 mg/kg dw) (Szumiało et al., 2013). The Hg level obtained in this study was also lower than those in birds from the Siberian Sea (4.01 mg/kg dw) (Kim et al., 1996) or the Barents Sea (11.1 mg/kg dw) (Savinov et al., 2003). On one hand, this probably resulted from the fact that gulls were residing in areas far from the Baltic Sea, where exploitation of Hg-contaminated fish was lower (Elmgren, 2001). On the other hand, it could be due to the exploitation of anthropogenic food from dumping sites (Meissner and Betleja, 2007). Trophic structure is a basic driver of variability in Hg biomagnification in the food chain. Larger piscivorous birds (in this case, grey herons and cormorants), for which trophic status is potentially the greatest, play a particular role. For livers of adult birds from this species, Hg levels of >10 mg/g dw were reported (Nam et al., 2005a; Misztal-Szkudlinska et al., 2011) which corresponds with our data. Other food web pathways important for Hg transfer are generally of lesser concern because the trophic status of the endpoint species is generally lower than that of piscivorous species. Benthic-based Hg transfer through invertebrates (included bivalves, insects, and their larvae) has been investigated using various diving species of waterfowl, contrary to transfer through the macrobenthos and plants, which are rarely analyzed (Wayland et al., 2001; Evers et al., 2005). It should be noted that water plants can be a source of Hg contamination (Szymanowska et al., 1999; Samecka-Cymerman et al., 2001; Peng et al., 2008). Hg transfer based mainly on vegetation and invertebrates concerned mainly the mallards examined in this study, which resulted in moderate hepatic Hg concentrations. Such results could also follow typical usage of anthropogenic food by an urban avian population (Figley and Van Druff, 1982). Similar hepatic Hg amounts for mallards were found by Kalisińska et al. (2013). This is consistent with studies from other authors, where lower levels of Hg were demonstrated in the livers of dabbling ducks than in diving ducks and sea ducks, since the latter two groups of ducks use benthic invertebrates (bivalves, crabs) and fishes (Wayland et al., 2001; Evers et al., 2005; Kalisinska et al., 2010).

The highest amounts of Se were found in the livers of large birds that specialized in catching other birds able to accumulate relatively high amounts of Hg, which was also confirmed by the studies of others (Horai et al., 2007; Nam et al., 2005a). At high exposures, both Se and Hg can be individually toxic, but their coaccumulation reduces each other's toxic effects. This mechanism applies to many species of wetlands vertebrates able to accumulate large amounts of Hg (Peterson et al., 2009). Moving away from the explicit specialization of food towards widening the food niche explains why the GRM analysis of rook did not reveal a statistically significant interaction between the hepatic concentrations of Se and Hg (Table 4). In the context of the above, it can be pointed out that there is a large variability of hepatic Se concentrations in the livers of birds in response to local conditions due to the availability of selenium. For the mallards examined in this study, the hepatic Se geometric mean was 5.5 mg/kg dw, which was similar to the value reported for other dabbling ducks, such as the blue-winged teal: 1.46 mg/kg ww (5.84 mg/kg dw) (Fedynich et al., 2007). Vest et al. (2009) reported a Se concentration of 3.74 mg/kg ww (14.96 mg/kg dw) and 2.77 mg/kg (11.08 mg/kg dw) for Northern shoveler livers collected from the Great Salt Lake (Utah, USA) during November and December, respectively. On the other hand, green-winged teals collected there in December had a Se concentration of 2.21 mg/kg ww (8.84 mg/kg dw). Sometimes high levels of Se were observed in diving ducks and sea ducks, for which benthic invertebrates are major prey items (Schummer et al., 2008). Common goldeneye *Bucephala clangula*, bufflehead *Bucephala albeola*, and long-tailed duck *Clangula hyemalis* wintering at Lake Ontario had the following Se hepatic concentrations: 12.0 mg/kg dw, 12.3 mg/kg dw, and 22.7 mg/kg dw, respectively (Schummer et al. 2010). In a study of sea ducks wintering in the Baltics, Pilarczyk et al. (2012) found 5.3 mg/kg dw, 7.2 mg/kg dw, and 6.6 mg/kg dw hepatic Se in the velvet scoter *Melanitta fusca*, the common scoter *Melanitta nigra*, and the long-tailed duck, respectively, values that are comparable with the data for mallards obtained in this study.

Similar to other authors, relatively high Cu concentrations were found here in the livers of wetland species of medium and high mass. Horai et al. (2007) observed high hepatic Cu levels in grey herons (mean: 791 mg/kg dw) and intermediate egret *Egretta intermedia* (mean: 787 mg /kg dw), but low levels in great white egret *Ardea alba* (173 mg/kg dw). In another group of grey herons, Horai et al. (2007) observed liver Cu levels of 127 mg/kg dw, while Kim and Oh (2013) only found 37.8 mg/kg dw in the same species. Schummer et al. (2011) observed high hepatic concentrations of Cu in mute swans ranging from 944 to 2441 µg/g dw, respectively, which can be explained by intensive water plant consumption. Mallards are generally omnivorous, but aquatic plants are an important food resource for them (Figley and Van Druff, 1982). Because of the wide range of occurrence of mallards, there are many comparative data from different parts of the world, such as for Spanish and Korean mallards (46.0 mg/kg dw and 40.3 mg/kg of Cu, respectively) (Mateo and Guitart, 2003; Kim and Oh, 2012). Median concentrations of Cu (39.49 mg/kg dw) in mallards from eastern Poland (Binkowski et al., 2013) were similar to the geometric mean concentration of Cu in mallards obtained within this study. On the other hand, relatively low Cu concentrations in livers of this duck (13.82 mg/kg dw) were reported by Mansouri and Majnoni (2014).

Functions of the antioxidant defense systems of birds are characterized by relatively high tolerance of elevated hepatic Cu and Zn levels (Koivula and Eeva, 2010), which can explain why water birds possess the ability to accumulate relatively high amounts of Cu and Zn. This was shown for copper in reports after the Aznalcóllar mine spill (Taggart et al., 2006). The geometric mean of hepatic Cu in the livers of common pochard *Aythya ferina* was 101.4 mg/kg ww (405.6 mg/kg dw) then, while for the red-crested pochard *Netta rufina* it was 134.51 mg/kg ww (538.04 mg/kg dw) (Taggart et al., 2006). In mallards, signs of Zn poisoning were not discerned until the hepatic concentrations were between 473 and 1990 mg/kg dw (Levengood et al. 1999) or 280 and 2900 mg/kg dw (Sileo et al., 2003). On the other hand, Carpenter et al. (2004) discovered that in trumpeter swan *Cygnus buccinator,* symptoms of Zn poisoning were associated with mining wastes and resulted in Zn liver concentrations of 154 mg/kg ww (616 mg/kg dw). Compared to the mallards tested in this study, Kim and Oh (2012) found low concentrations of Zn (95.5 mg/kg dw). On the other hand, ducks may accumulate more Zn. Nam et al. (2005b) found average Zn values of 236 mg/kg dw, clearly exceeding our values for mallards. High hepatic concentrations of Zn (259 mg/kg dw) were noted in the spot-billed duck, which can be explained by Zn binding to many proteins, including metallothioneins, and also by Zn's role in controlling processes such as metalloenzyme activation, oxygen transport, and redox activities in birds. This indicates that species-specific cellular constituents may be responsible for Zn accumulation (Nam et al. 2005b).

Kim and Oh (2012) and Mansouri and Majnoni (2014) reported the following decreasing sequence of element concentrations for the livers of mallards: Zn > Cu > Pb > Cd. Kalisinska et al. (2004) confirmed this sequence for adult birds, although it was found that at one nesting location Cd concentration prevailed over Pb in immature birds. A similar conclusion was also drawn by Binkowski and Meissner (2013) in the analysis of duck blood. In the mallards analyzed in this study, Cd concentrations were higher than Pb. The above sequence of hepatic element concentrations was also found in the Eurasian woodcock and grey heron (Kim and Oh, 2013). Among the remaining 13 species studied by us, 8 showed the above sequence of concentrations.

As shown by RDA, foraging and nesting in wetlands (Figure 2; Table 7) favored the accumulation of manganese. Other authors (Nam et al., 2005a; Horai et al., 2007) also showed that hepatic concentrations of Mn were higher in piscivorous birds than herbivorous and omnivorous species. The range of Mn concentrations for the great cormorant as well as the mean hepatic concentration for grey heron corresponded with those given by other authors: 19.0 mg/kg dw, 16.6 mg/kg dw (Nam et al., 2005a, 2005b), and 15.7 mg/kg dw (Horai et al., 2007) for the cormorant and 13.4 mg/kg dw for the grey heron (Horai et al. 2007). The relatively high Mn concentrations in mallards obtained in this study were complementary to those reported by Nam et al. (2005b): 13.7 mg/kg dw.

In conclusion, in the present study, it was found that hunting pressure in the form of lead bullets can affect mainly terrestrial birds, such as corvids. Expanding food niches in the direction of being omnivorous reduces the accumulation of Zn, Cu, Se, and Hg. Accumulation of these elements was favored by specialization in capturing fish. Consumption of soil invertebrates can promote the accumulation of Cd. The relatively broad food niche for rooks prevents excessive accumulation of certain elements.

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