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Trace element residues in eggshells of Grey Heron (*Ardea cinerea)* from colonies of East Poland

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Abstract. Concentrations of 18 trace elements in the eggshells of grey heron (*Ardea cinerea*) from four colonies located in the Lublin region (East Poland) were determined. The pattern of element concentration in grey heron eggshells for all colonies followed the order: Si > Sr > B > Al > Zn > Fe > Ba > Li > Cu > Mn > Se > As > Cr >Ni > Pb > Sc > V > Cd. The results on the concentrations of elements in water sediments showed different accumulation pattern such as: Mn > Sr > Ba > Zn > Cr > Pb > Cu >Ni> As > Cd >Al. On the other hand, an increasing concentrations of the following toxic element sequence: Cr > Pb > Cd was found in both cases. This phenomenon was observed in three out of four grey heron colonies. On average, the eggshells contained 140 mg/kg dw (dry weight) of strontium. The maximal concentration of Sr reached a level up to 203 mg/kg dw while the level of aluminium was up to 18.0 mg/kg dw. Geometric means found for Li, Cu, Ni, Pb and Cd in eggshells from all colonies were 2.05, 2.04, 0.53, 0.47 and 0.06 mg/kg dw respectively. Our study showed that herons can be a good bioindicator of elements such as Sr, Al, Cr, Pb and Cd.

Key words: grey heron, eggshells, trace elements, aluminium, strontium, accumulation.

Introduction

Different groups of animals (Caro 2010, Gregory & van Strien 2010, Markovic et al. 2012, Stankovic et al. 2013, Riani et al. 2014) including birds are used for monitoring of wetlands due to the particular role they perform as ecosystem linkers (Sekercioglu, 2006). In Europe, herons, especially grey herons (*Ardea cinerea*) are the main species nesting in colonies, which are feeding on a variety of aquatic organisms (Jakubas & Mioduszewska 2005, Gwiazda & Amirowicz 2006). Being the top predators, they may accumulate many non-persistent and persistent environmental contaminants, including heavy metals. Their longevity, mobility, colonial life and social foraging make them a useful tool in the monitoring of aquatic environments (Ayas 2007, Komosa et al. 2009).

Most recent studies emphasise that one of the basic mechanisms in the detoxification of the bird organisms is the excretion and deposition of heavy metals, for example in feathers (Bostan et al. 2007, Malik & Zeb 2009, Kim & Koo 2008, Skorcic et al. 2012). Females, in contrast to males, can eliminate heavy metals by their accumulation in the material of eggshells. Such a detoxification mechanism is especially important, owing to the fact that heavy metal deposition inside the egg may lead to the atrophy of embryo. The role of the avian eggshell in sequestering inorganic elements is still not clear. The eggshells are mainly composed of calcium and magnesium carbonates (Zohouri et al. 1998, Hunton 2005, Mora et al. 2011). Some researchers found that trace metals such as lead, cadmium and copper may interact with the metabolic pathway of calcium and magnesium, therefore, the toxic elements can be incorporated more easily in the eggshell (Bokori et al. 1995). Only a few reports exist on heavy metals (mostly lead and cadmium) accumulation in eggshells (Burger 1994, Nyholm 1998, Borera et al. 1997).

The purpose of our study was to determine the levels of concentration of trace elements in eggshells collected in four colonies of grey heron in East Poland and to compare and discuss them with the accessible data on other water birds species. The aim was also to analyse the data against the pattern of accumulation of the metals in nearby river sediments.

Materials and Methods

Analysed material consisted of the eggshells of grey heron, collected in four heronaries: Chodlik (N 51°12´40, E 21°55´5), Kosmów (N 50°42´32, E 24°0´29), Małoziemce (N 51°21´46, E 23°36´51) and Wólka Michowska (N 51°33´20, E 22°21´27) of Lublin region (East Poland).

The examined heronaries are located near the main rivers of East Poland (Chodlik - near the Vistula river, Kosmów – near the Bug river, Małoziemce and Wólka Michowska – near the Wieprz river). In South East Poland yet in seventies of the previous century a very high number of grey heron colonies was localized near fish ponds. However, the intensive shooting of birds foraging on ponds brought about the atrophy of these colonies (Kitowski & Krawczyk 2005). Shooting pressure makes the grey herons to avoid foraging on water reservoirs and ponds and preferring big rivers and their basins. At present the highest number of grey heron nests in South Poland is localized in colonies near the biggest rivers of this region. Colonies were chosen for experiments on the basis of being representative of the above mentioned processes and belong to the biggest in the Lublin region. During the period of experiments (2012) the examined colonies consisted of the following numbers of nests: Małoziemce - 48 nests, Chodlik - 34 nests, Kosmów - 82 nests, Wólka Michowska - 71 nests.

The available data on water sediments from this region (Rogulska & Grzywaczewska 2012) indicate that, occasionally, the elevated level of strontium resulted from natural geological factors might be found on the Bug River (Małoziemce, Kosmów colonies). On the other hand, on Vistula (Chodlik colony) and Wieprz (Wólka Michowska) rivers in several Lublin region collection points, the geochemical background was exceeded by Cd, B, Ni, Pb and Zn which indicates their anthropogenic emission to water. Vistula is the largest river of Poland while Wieprz collects the ground waters from the whole Lublin region (see map on Fig. 1).

Eggshells (entire eggshells or their fragments) were collected at the end of April 2012 under different trees to ensure the representativeness of eggshell samples, as grey herons are colonial breeders. Usually 10-20 eggshells (including their fragments) from each colony were collected. Six eggshells from each heronary were chosen at random and subjected to cleaning and the preparation of 2 samples from each eggshell (total number of samples per heronry amounted 12). The procedure required the sample to be soaked in demineralised water (ELGA Pure lab Classic) for 5 minutes, cleansed mechanically with a wet soft brush, dried at room temperature on a cheesecloth and stored in PVC containers at 4°C before further use.

All glassware and utensils were rinsed with tap water, soaked in an acid bath (5M HNO_3) for a minimum of 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. Samples were ground in a ceramic mortar. Weighted portions of the samples 500 ± 1 mg were poured with 10mL of concentrated HNO_3 (Sigma Aldrich) 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 (Berghof Speedwave) in Teflon vials (DAP 100 type). Mineralization process has run according to the following scheme: 15 min. from the room temperature to 140°C, 5 min. at 140°C, 5 min. from 140°C to 170°C, 15 min. at 170°C, cooling to room temperature (variable time). The pressure

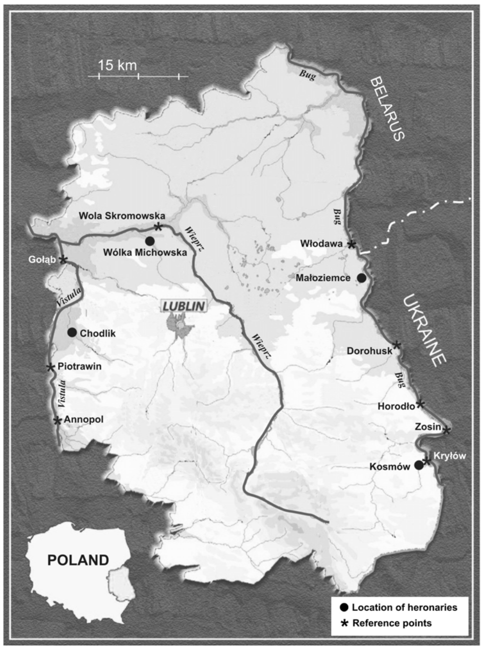

Figure 1. Location of heronries and reference points.

measured over the whole mineralization process did not exceed the value of 12 bar. A clear acidic solution was obtained after completion of the mineralization process. Solutions cooled to the room temperature were transferred to a 50mL volumetric flask and filled up with a demineralised water to the indicated level.

In this study, ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) from Thermo Scientific iCAP Series 6500, equipped with a charge injection device (CID) detector was used for the determination of elements. Control of the spectrometer was provided 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 - 0.4 L min^{-1}, max integration times - 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:

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

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 ppm

C) Analityk – ^{11}B, ^{137}Ba, ^{7}Li, ^{32}S, ^{28}Si in 5% HNO_3 - 40 ppm (prepared from single-element stock solutions -1000 ppm).

Detection limits were established by measuring a blank solution (5 % HNO_3). The solution was analyzed seven times with each analysis having three replicates, the mean of 3x the standard deviation value from all of the runs was calculated. Results obtained are given in Table 1.

The analytical procedure was checked using Quality Control Standard (QCS-28) from Inorganic Ventures (Sc element was not certified). The accuracy ranged between 93 and 112%.

The data concerning the pattern of concentration of 11 elements (Al, As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sr, Zn) found in the grey heron eggshells examined were compared with the respective concentrations of those metals in the river sediments which were collected in the points distant up to 25 km away from grey heron breeding colonies. The sediments were collected in late autumn, 2011. The sediments collection points were chosen from a database, based on the information about the home range of the examined species. The most reliable data in this matter engaging radio-tracking studies of birds (Marion 1989), showed that grey herons are able to forage in the distances up to 38 km from their habitats with the mean distance of 20 km. Based on this information the appropriate reference points from a database were chosen. The location of the examined heronaries and the sediments collection points (reference points) are shown on Fig.1.

The following collection (reference) points for the respective, examined colonies were used:

- Chodlik colony (river Vistula) - Piotrawin (E 21.79681, N 51.11306), Gołąb (E 21.8725, N 51.47667) and Annopol (E 21.83556, N 50.88306)
- Wólka Michowska colony (river Wieprz) - Wola Skromowska (E 22.45806, N 51.61194).
- Małoziemce colony (river Bug) - Włodawa (E 23.56639, N 51.55139) and Dorohusk (E 23.82344, N 51.16181)
- Kosmów (river Bug) - Krylów (E 24.06153, N 50.68272), Zosin (E 24.145, N 50.86528) and Horodło (E 24.04004, N 50.83952).

For the examined colony, the mean concentration calculated from the respective data of the above reference points were used for data analysis.

All the statistical calculations were carried out using STATISTICA PL Software. Geometric means as well as median values were calculated and the differences between the data from different locations were analyzed. Differences among medians were tested by the ANOVA Kruskall-Wallis *H*-test and by non-normal data distribution.

Table 1. Detection limits of ICP-OES method established by measuring a blank solution (5 % HNO_3). The solution was analyzed seven times with each analysis having three replicates, the mean of 3x the standard deviation value from all of the runs was calculated.

Element	Wave length [nm]	Plasma View	Limit of detection (LOD) [ppm]
Al	396.152	Radial	0.021
As	193.759	Axial	0.011
B	208.959	Radial	0.010
Ba	413.066	Radial	0.014
Cd	214.438	Axial	0.001
Cr	267.716	Axial	0.003
Cu	324.754	Axial	0.002
Fe	259.837	Radial	0.021
Li	670.784	Axial	0.040
Mn	259.373	Axial	0.002
Ni	221.647	Axial	0.001
Pb	220.353	Axial	0.010
Sc	361.384	Axial	0.002
Se	196.090	Axial	0.012
Si	212.412	Radial	0.011
Sr	421.552	Axial	0.003
V	292.402	Axial	0.003
Zn	206.200	Axial	0.010

Results

Table 2 shows the concentrations of the trace elements (dry weight, dw) in the eggshell samples collected in the four examined heronaries. The pattern of elements concentration in grey heron eggshells for all colonies followed the order: Si > Sr > B > Al > Zn > Fe > Ba > Li > Cu > Mn > Se > As > Cr > Ni > Pb > Sc > V > Cd. The results on the concentrations of water sediments show different accumulation pattern such as: Mn > Sr > Ba > Zn > Cr > Pb > Cu >Ni > As > Cd >Al. On the other hand, an increasing concentrations of the following toxic element sequence: Cr > Pb > Cd was found in both cases. This phenomenon was observed in three out of four grey heron colonies (except that in Małoziemce) (Table 2). The geometric mean concentrations of Cr in the examined eggshells amounted to 0.78 mg/kg dw but the concentration of this element in the bird colony from the Małoziemce area was a few times lower as compared to the other locations (Table 2). The eggshells contained 140 mg/kg dw of Sr, on average. The maximal level of Sr was found in the Chodlik heronary (Table 2). The concentrations for many elements showed noticeable variability between the colonies of heron. The highest variability was found for such elements as Zn, Ni, Cr, Pb and Cd. In the case of Li, Sr, Fe, Cu and Al differences in the median concentrations turned out to be the smallest (Table 2). In the eggshells from the Chodlik area, the highest median concentrations of several elements having an adverse influence on the embryo development (Ni, Cd, Cr, Al, Cu and Sr) were found (Table 2). Samples from the colony from the Małoziemce area contained the highest median concentrations of As, Ba, Mn and Pb but, on the other hand, they revealed the lowest median concentrations of Cu, Ni, Li, B, Cd, Fe

Table 2. Concentrations of elements (in mg/kg dry weight) in the grey heron eggshells from 4 studied colonies. Mean (±SD) and medians, ranges of measurements given below. Statistical differences between colonies estimated by using Kruskall-Wallis H-test; df =3, N=12 for each colony. *P < 0.05.**P < 0.01. ***P < 0.001.

Elements	Chodlik		Kosmów		Wólka Michowska		Małoziemce		Data for all colonies		H
	mean	median	mean	median	mean	median	mean	median	geometric mean	median	
Si	303 (63.9) 234.0-409.0	293	294 (40.30) 234.0-346.0	295.0	272 (45.70) 203.0-329.0	274.0	271 (25.90) 231.0-303.0	279.0	282.0	279.0	1.647
Sr	202 (42.50) 139.0-255.0	205	161 (61.30) 89.70 - 222.0	170.0	103.0 (20.0) 73.58 -134.80	101.0	130 (19.50) 119.0-169.0	123.0	140.0	130.0	11.592*
B	70.97 (36.0) 39.0-124.0	57.1	43.7 (9.85) 32.20-60.50	43.0	55.6 (24.10) 30.10-98.0	46.5	21.9 (26.80) 1.91-66.47	9.51	33.1	44.4	6.673
Al	18.0 (3.56) 14.90-22.90	17.8	12.3 (1.41) 9.80 -13.60	12.5	12.0 (1.65) 10.60-14.20	11.2	15.2 (6.40) 9.39-26.60	13.5	13.8	13.5	9.327*
Zn	15.0 (0.82) 13.90-16.10	14.9	9.49 (1.94) 6.81-11.70	10.3	19.9 (3.55) 16.60-26.20	19.5	8.02 (1.50) 5.42-9.57	8.42	12.1	12.8	19.980***
Fe	13.5 (6.76) 9.64-27.10	10.7	9.38 (1.84) 7.54-12.90	9.01	9.07 (1.02) 8.27-11.02	8.80	7.88 (7.49) 3.11-22.80	5.21	8.92	9.09	10.527*
Ba	2.71 (1.72) 1.55-4.79	2.57	3.73 (1.20) 2.26 - 5.72	3.41	2.74 (0.73) 1.76-3.58	2.73	6.82 (4.44) 1.65-11.71	7.50	3.33	3.07	3.627
Li	1.88 (0.03) 1.81-1.85	1.89	2.88 (1.55) 1.90-5.57	1.96	1.92 (0.04) 1.87-1.97	1.92	1.87 (0.01) 1.86-1.88	1.88	2.05	1.89	10.860*
Cu	2.57 (0.50) 1.88-3.40	2.46	2.20 (0.33) 1.70- 2.64	2.20	2.29 (0.70) 1.55-3.45	2.11	1.47 (0.39) 1.11-2.06	1.26	2.04	2.09	10.447*
Mn	1.85 (0.50) 1.36-2.67	1.68	2.05 (1.06) 0.83-3.46	1.84	1.29 (0.33) 0.89-1.75	1.27	1.82 (1.09) 0.50-3.34	1.89	1.57	1.53	2.393
Se	1.15 (0.05) 1.07-1.20	1.16	1.11 (0.07) 1.03-1.18	1.11	1.14 (0.19) 0.85-1.31	1.18	1.12 (0.03) 1.07-1.15	1.12	1.12	1.14	1.020
As	0.89 (0.08) 0.79-1.00	0.91	0.88 (0.07) 0.79-0.98	0.90	0.94 (0.07) 0.87-1.03	0.90	1.09 (0.21) 0.83-1.37	1.05	0.94	0.92	5.487
Cr	1.12 (0.02) 1.09 -1.13	1.12	1.09 (0.05) 1.02-1.15	1.10	1.07 (0.03) 1.03-1.12	1.07	0.29 (0.04) 0.24-0.34	0.29	0.78	0.90	15.807***
Ni	0.98 (0.13) 0.74-1.14	1.02	0.64 (0.03) 0.61-0.69	0.63	0.68 (0.08) 0.61-0.83	0.66	0.19 (0.04) 0.14-0.24	0.17	0.53	0.65	19.567***
Pb	0.47 (0.16) 0.47-0.73	0.47	0.64 (0.43) 0.33-1.37	0.40	0.28 (0.07) 0.17-0.36	0.30	0.76 (0.35) 0.59-1.47	0.63	0.47	0.43	12.487**
Sc	0.08 (0.01) 0.07-0.09	0.08	0.08 (0.01) 0.07-0.08	0.08	0.08 (0.01) 0.07-0.09	0.08	0.08 (0.01) 0.08-0.09	0.08	0.08	0.08	0.440
V	0.07 (0.01) 0.05-0.11	0.07	0.08 (0.01) 0.05-0.11	0.08	0.07 (0.02) 0.03 -0.10	0.07	0.07 (0.01) 0.06-0.09	0.07	0.07	0.08	2.420
Cd	0.07 (0.01) 0.06 -0.08	0.07	0.06 (0.01) 0.05-0.07	0.06	0.05 (0.01) 0.05-0.06	0.05	0.05 (0.01) 0.04-0.08	0.05	0.06	0.06	11.970**

Zn, as compared to the other colonies (Table 2).

In the case of several elements, the concentration show high variability both within and between the colonies, which brought sharp contrasts to the data obtained for particular elements. No statistical differences were found for boron content in the analysed eggshells among studied colonies.

Discussion

In the presented work concentrations of metals in the eggshells of grey heron were measured.

Geometric mean is the arithmetic average of a group of data values, therefore it is sensitive to extreme scores when population samples are small (12 samples per colony in this study). Medians are less sensitive to extreme scores and are more representative of the central tendency of the sample set, being a better indicator of the variability where the middle of the class is achieving. The mean content of Cu in the eggshells of the colonial water birds such as grey heron, night heron (*Nycticorax nycticorax*) and Audouin's gull (*Larus audouini*) nesting in Turkey was 6.75 and 1.37 mg/kg dw for the first two species, and 1.85 and 10.20 mg/kg dw for the gull species (two values concerns two breeding colonies) (Ayas 2007, Ayas et al. 2008). However, the eggshells of the grey heron nesting in three different colonies in North Poland on the areas considered as unpolluted had an average level of Cu ranging between 4.13 and 5.27 mg/kg dw and of Zn ranging from 1.62 to 2.24 mg/kg dw (Dmowski 1999). Our data concerning Cu, ranging from 1.11 to 3.45 mg/kg dw (mean value 2.05), directly correspond with the above mentioned results, contrary to the data obtained for Zn, where much higher levels than reported previously were found. It is valuable to compare the data of other experiments done on the other colonial water birds such as egrets, gulls and terns, even without having a common thread. The reason is that these species have similar feeding habits and explore similar water habitats.

Lam et al. (2005) showed Zn concentrations as high as 6.29 mg/kg dw, 6. 58 mg/kg dw and 3.92 mg/kg dw, for the eggshells of little egret (*Egretta garzetta*), black-crowned night heron (*Nycticorax nycticorax*), and bridled terns (*Sterna anaethetus*), respectively, nesting near Hongkong. Those Zn concentrations were 6.3, 7.8 and 12 times higher, respectively, when compared to Cu concentrations. Such observation stays in accordance to our results, where geometric means of Zn concentrations for all the collected eggshells were *c.a.* 6 times higher than those of Cu. Dauwe et al. (1999) found that the mean Zn and Cu concentrations were higher in the egg alone than in the eggshell. Dauwe et al. (1999) also found important differences of Zn and Cu levels in the eggshell collected on the polluted area and the reference site (considered as unpolluted one). In the egg content, however, the values did not differ markedly between polluted and unpolluted areas, indicating that Zn and Cu concentrations are probably homeostatically regulated inside the egg. Zhou et al. (2005) analysed the concentrations of the residues of heavy metals in eggshells of four heron species: black-crowned night heron, little egret, Chinese pond heron (*Ardea bacchus*) and cattle egret (*Bubulcus ibis*) from the Anhui province in East China, where the sequence of decreasing concentrations of Cr > Pb > Cd was found. A similar sequence of concentrations of toxic elements was confirmed by Lam et al. (2005) for little egrets, black-crowned night herons and bridled terns.

The data on grey heron presented in this study confirmed such a sequence of concentrations at three out of four colonies, as it is seen in Table 2. On the contrary, data obtained from the eggshells of six different heron species nesting in Barak Valley (Assam, East India) showed a different sequence of concentrations of toxic elements, such as: Pb > Cr > Cd (Dev et al. 2010). For the eggshells of little egret, Hashmi et al. (2012) showed that the pattern of metal concentration followed the order: Pb > Cd > Cr. Additionally, Ayas (2007) showed that the eggshells of grey heron from Nallihan Bird Paradise (Ankara, Turkey) contained lower concentrations of Cd than Pb. The concentration ratio of these elements amounted to 1:7.3 (values: 0.93 mg/kg dw to 6.83 mg/kg dw). We found a very similar proportion of the concentrations of Cd and Pb amounting 1:7.8 for all the examined eggshells of grey heron (Table 2). However, in the case of night herons, nesting sympatrically with grey herons, a ratio of 1:4.82 was demonstrated by Ayas (2007) between Cd (0.23 mg/kg dw) and Pb (1.11 mg/kg dw). Interestingly, both surveys on grey herons conducted in Poland and in Turkey (Ayas, 2007) showed that the accumulation of Cu in the eggshells was higher as compared to Ni. In the present study, a ratio of 1.0:3.85 of Ni and Cu was found (see Table 2), while for the eggshells from Turkish heronary it was as high as 1.0:16.7.

The group of Hashmi et al. (2012) studying the eggshells of the little egret, also demonstrated a higher level of accumulation of Cu in comparison with Ni, and showed the respective proportion of these elements being 1.0:1.82.

Sr is the element commonly considered as safe for birds' breeding. However, some authors draw attention to its embriotoxic action (Moon 1994, Mora et al. 2007) as it can affect the transportation of calcium from the eggshell to the embryo, leading to embryo deformations (Elaroussi & Deluca 1994).

Concerning Al, the available reports indicate an adverse impact of this element on birds. Nyholm (1981) suggested that Al could interfere with the phosphate metabolic pathways and thereby reduce the availability of Ca needed for eggshell formation. However, some further studies have not supported this hypothesis. Miles et al. (1993) wrote that starlings (*Sturnus vulgaris*) fed with a diet rich in aluminium tended to have higher Al levels in bones (femur), but no defects of eggshells were found, as suggested by Nyholm (1981). Yet the results of the experiments carried out by Zohouri et al. (1998) showed that dietary Al supplementations may have negative effects on eggshell quality. Uyanik et al. (2008) showed that dietary Al supplementations can have adverse effects on egg quality, except for eggshell thickness.

There are only few reports on boron content in birds eggshells but it was found that the shell thickness was not affected by dietary supplementation with this element (Rossi et al. 1993, Mizrak et al. 2010). No statistical differences were found for B content in the analysed eggshells ($P \leq 0.05$) of grey heron in this study (Table 2). The content of this element was definitely higher than that demonstrated for other bird species (Al-Obaidi et al. 2012).

Several elements (Cr, Ni, Zn, $P \leq 0.001$; Pb, Cd, $P \leq 0.01$) show high variability within the colony as well as between them, which brought sharp contrasts to the data obtained for particular elements. Similar effects of high individual variability of the concentrations of several elements were observed for other colonial water birds (Boncompagni et al. 2003, Misztal-Szkudlinska et al. 2011, Skorcic et al. 2012). The above-mentioned variability of concentrations can be explained in terms of the high diversity of areas used by individual female herons to forage (Ayas 2007). Different concentrations of several elements in eggshells collected in a defined colony can be explained in terms of the different degrees of contamination in the grey heron preying area expressed by home range. Therefore, the interpretation of the data obtained during a study of colonial water birds should take into account the results of direct field observations of the predation areas preferred by birds. Such a dependency is clearly seen for the grey heron colony from the Małoziemce area, where some birds forage in the bed of the Bug river and its wetlands, and some in dystrophic forest lakes of the Sobibór Forest complex (protected as a part of the Sobibór Landscape Park).

In spite of a short distance from fish ponds, the grey herons nesting in the Wólka Michowska and Chodlik areas do not forage there as the result of shooting. Instead, they use riverbeds and wetlands of the rivers Wieprz and Vistula 10-15 km away (Kitowski & Krawczyk 2005, Kitowski I. – unpublished data).

The concentrations of 11 elements in the eggshells measured in this study were analysed against the data obtained from the Regional Environment Protection Inspectorate in Lublin concerning the respective element concentrations in the sediments of rivers where the foraging grey herons were seen (Table 3). A direct comparison of these data set is not possible as they concern different matrices. Although, the thorough analysis of the metal level patterns can give some information on the possible connection between the state of the environment and the heavy metal accumulation pattern in the eggshells. Only two elements (Al and Sr) showed a much higher accumulation rate in eggshells as compared to the river sediments (see Tables 2 and 3). The eggshell/sediment ratios of Sr concentrations in the examined heronaries amounted to, respectively: Wólka Michowska: - 4.0, Chodlik - 3.5, Małoziemce - 2.5 and Kosmów - 1.0. In the case of Al the relevant ratios were equal to: 48, 46, 29 and 26 for Wólka Michowska, Małoziemce, Kosmów and Chodlik, respectively. The concentrations of remaining elements turned out to be higher in the river sediments in comparison with the grey heron eggshells (Table 2 and 3). Analyses of Sr from this study correspond with the data published previously for other water bird species (Mora 2003, Mora et al. 2007). The mean concentrations of Sr up to 1505 mg/kg dw were found for Yellow-breasted chats (*Icteria virens*) and 1002 mg/kg dw for Brown-headed cowbirds (*Molothrus ater)* from Arizona. Such a results were interpreted in terms

Table 3. Concentrations mean (±SD) of selected elements (in mg/kg dry weight) in the river sediments near the studied colonies of the grey heron. Names of rivers are given in brackets below colony names. Ranges of measurements are listed below.

Element	Chodlik (Wisła)	Małoziemce (Bug)	Kosmów (Bug)	Wólka Michowska (Wieprz, Tyśmienica)
Mn	729 (428) 241-1040	219 (18.4) 206-232	512 (207) 310-723	317 (45.3) 285-349
Sr	57.7 (18.8) 36.0-69.0	53.5 (7.8) 48.0-59.0	158 (42.5) 125-206	25.7 (10.3) 18.4-33.0
Ba	71.7 (33.5) 33.0-92.0	28.5 (0.71) 28.0-29.0	56.3 (20.5) 35.0-76.0	36.0 (7.01) 31.0-41.0
Zn	147 (79.4) 55.0-194	13.0 (0.05)* -	27.0 (9.54) 18.0 - 37.0	28.6 (19.0) 15.1-42.0
Cr	17.7 (7.6) 9.0-23.0	5.5 (0.71) 5.0-6.0	11.0 (2.65) 9.0-14.0	7.23 (5.33) 3.46-11.0
Pb	21.7 (12.9) 7.0-31.0	3.5 (0.71) 3.0-4.0	6.0 (1.0) 5.0-7.0	7.40 (6.52) 2.79-12.0
Cu	19.7 (11.0) 7.0-26.0	4.5 (0.71) 4.0-5.0	7.67 (2.08) 3.03-7.0	5.01(2.81) 3.03-7.0
Ni	20.0 (8.9) 10.0-27.0	4.0 (0.05)* -	8.0 (2.0) 6.0-10.0	3.5 (2.13) 1.99-5.0
As	5.3 (3.1) 2.0-8.0	2.0 (0.05)* -	3.0 (1.00) 2.0-4.0	1.75 (0.35) 1.5-2.0
Cd	1.1 (0.8) 0.25-1.6	0.3 (0.01) 0.25-0.3	0.25 (0.05)* -	0.92 (0.94) 0.25-1.6
Al	0.68 (0.23) 0.41-0.82	0.33 (0.03) 0.3-0.4	0.43 (0.05) 0. 37-0.46	0.25 (0.06) 0. 20-0. 29

Concentrations determined by the Polish Geological Institute, according to an internal standard (PIG/2009 – ICP-AES/OES) and provided by the Regional Environment Protection Inspectorate of Lublin. *- Limit of detection (LOD)

of a response to a high strontium deposits at nesting habitats. Interestingly, the river sediments within the research area showed concentrations of strontium between 44 - 230 mg/kg dw (Mora 2003, Mora et al. 2007).

Contrary to other authors, we have not observed bioaccumulation of Ni, Cu or Pb in the grey heron eggshells. Birds nesting at the Nallihan Bird Paradise showed the eggshell to sediment ratios of Ni, Cu and Pb concentrations being as high as 1.002, 19.630 and 22.909, respectively (Ayas 2007).

The correlations between the elements mean concentrations in the eggshells versus the respective mean concentrations of elements in sediments were calculated (see Table 4). The weak correlations between most of the element concentrations were observed. A relatively strong correlations were found in the case of Sr (R^2= 0.885) and Al (R^2=0.630).

Table 4. Analysis of correlations between the mean concentrations of elements in the examined eggshells and water sediments (R- correlation coefficient (Pearson's), R^2- coefficient of determination)

Element	R	R^2
Mn	0.416668	0.173612
Sr	0.940668	0.884855
Ba	-0.63693	0.405686
Zn	0.304006	0.092419
Cr	0.639022	0.408349
Pb	-0.38497	0.148203
Cu	0.692956	0.480188
Ni	0.759331	0.576583
As	-0.56423	0.318351
Cd	0.421919	0.178016
Al	0.793288	0.629306

In conclusion we point out that eggshells of grey herons appear to be good bioindicators for the monitoring of river valleys concerning toxic elements such as Sr, Al, Cr, Pb and Cd. Concentrations of Sr and Al in the eggshells were multiple times higher than those in the river sediments. Our study provided data confirming the fact that the eggshells are an important excretion reservoir of nonessential inorganic elements (particularly Al and Sr) for a female of grey heron. Additionally the concentrations of these elements were strongly correlated with the respective concentrations of these elements in water sediments. Also, an increasing concentrations of the following toxic element sequence: Cr > Pb > Cd was found in both: eggshells and water sediments. This phenomenon was observed in three out of three grey heron colonies. The interpretation of level of various element concentrations in the eggshells of grey herons (as well as other colonial water birds) requires consideration of the presence of these elements in the foraging areas preferred by birds. However, the important factor in case of colonial water birds including herons is the utilization of unpredictable food sources (Marion 1989, Jakubas & Mioduszewska 2005, Gwiazda & Amirowicz 2006) which fluctuate spatio-temporally and thus may influence diversification in the process of accumulation of some elements in the birds tissues.

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