
Published in 36 issues each year
Volume 490 • 15 AUGUST 2014 • ISSN 0048-9697
ELSEVIER
Science OF THE Total Environment
Anthroposphere
Biosphere
Atmosphere
TOTAL ENVIRONMENT
Hydrosphere
Lithosphere

Science of the Total Environment 490 (2014) 1057–1064

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Residues of chromium, nickel, cadmium and lead in Rook *Corvus frugilegus* eggshells from urban and rural areas of Poland

Grzegorz Orłowski [a,*], Zbigniew Kasprzykowski [b], Wojciech Dobicki [c], Przemysław Pokorny [c], Andrzej Wuczyński [d], Ryszard Polechoński [c], Tomasz D. Mazgajski [e]

[a] *Institute of Agricultural and Forest Environment, Polish Academy of Sciences, Bukowska 19, 60-809 Poznań, Poland*
[b] *Department of Ecology and Nature Protection, Siedlce University of Natural Sciences and Humanities, Prusa 12, 08-110 Siedlce, Poland*
[c] *Department of Limnology and Fishery, Wrocław University of Environmental and Life Sciences, Chełmońskiego 38C, 51-630 Wrocław, Poland*
[d] *Institute of Nature Conservation, Polish Academy of Sciences, Lower-Silesian Field Station, Podwale 75, 50-449 Wrocław, Poland*
[e] *Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679 Warszawa, Poland*

HIGHLIGHTS

- Concentrations of Cr, Ni, Cd and Pb are reported for Rook eggshells from 43 rookeries.
- Cr, Ni and Pb levels were significantly higher in urban than in rural areas.
- Bioaccumulation of Cr, Ni and Pb suggests a pollution gradient (urban > rural areas).
- Females rapidly bioaccumulate Cr, Ni and Pb in breeding areas.
- No difference found for Cd levels, which are probably regulated physiologically.

ARTICLE INFO

Article history:
Received 9 March 2014
Received in revised form 23 May 2014
Accepted 23 May 2014
Available online 9 June 2014

Editor: Charlotte Poschenrieder

Keywords:
Non-essential elements
Toxic metals
Agricultural contaminants
Soil contamination
Trace elements
Soil-invertebrate diet

ABSTRACT

We examined the concentrations of chromium (Cr), nickel (Ni), cadmium (Cd) and lead (Pb) in Rook *Corvus frugilegus* eggshells from 43 rookeries situated in rural and urban areas of western (= intensive agriculture) and eastern (= extensive agriculture) Poland. We found small ranges in the overall level of Cr (the difference between the extreme values was 1.8-fold; range of concentrations = 5.21–9.40 Cr ppm), Ni (3.5-fold; 1.15–4.07 Ni ppm), and Cd (2.6-fold; 0.34–0.91 Cd ppm), whereas concentrations of Pb varied markedly, i.e. 6.7-fold between extreme values (1.71–11.53 Pb ppm). Eggshell levels of these four elements did not differ between rural rookeries from western and eastern Poland, but eggshells from rookeries in large/industrial cities had significantly higher concentrations of Cr, Ni and Pb than those from small towns and villages. Our study suggests that female Rooks exhibited an apparent variation in the intensity of trace metal bioaccumulation in their eggshells, that rapid site-dependent bioaccumulation of Cu, Cr, Ni and Pb occurs as a result of the pollution gradient (rural < urban), and that Cd levels are probably regulated physiologically, even though these were relatively high, which could be treated as an overall proxy of a heavy Cd load in the soil environment.

1. Introduction

Populations of wild animals living in farmed landscapes are especially susceptible to the adverse or even lethal effects of agriculturally related chemicals. Animals acquire these chemicals primarily from pesticides, fertilisers or food additives used in livestock production, through the diet and respiration (cf. Malmberg, 1973; Pinowski et al., 1994; Ramey and Sterner, 1995; Zhuang et al., 2009; Castilla et al., 2010), but local point sources of anthropogenic pollution, such as smelters or mining activities, may be of greater importance (Dmowski and Golimowski, 1993). The recent increase in agricultural productivity in temperate regions (Tilman et al., 2002) is closely related to the use of larger amounts of agrochemicals, which enter the environment and are found mainly in soil and water (Burger, 2008). In the 20th century, agricultural activity led to an apparent increase in environmental pollution, especially in the concentrations of toxic metals and agrochemicals in arable topsoil. For instance, cadmium levels in agricultural soils in Sweden increased by approximately 0.05% per year (Olsson et al., 2005). The main sources of cadmium and lead in arable soils are fertilisers: organic fertilisers (farmyard manure and slurry) can contain residues of feed components, and

* Corresponding author. Tel./fax: +48 65 513 42 53.
E-mail address: orlog@poczta.onet.pl (G. Orłowski).

http://dx.doi.org/10.1016/j.scitotenv.2014.05.105
0048-9697/

mineral fertilisers (of primarily phosphate rock origin) may have an extremely high content of these metals (Linden et al., 2001; Dach and Starmans, 2005). An increase in pesticide use, the livestock numbers and the improper utilisation of huge amounts of animal faeces containing copper and zinc derived from food additives are the main threats ensuing from the intensification of agricultural productivity (de Vries et al., 2002; Dach and Starmans, 2005).

Soil-invertebrate-feeding bird species or individuals with a high fraction of such prey types in their diets, such as the Rook *Corvus frugilegus*, are particularly vulnerable to high doses of toxic chemicals (Malmberg, 1973; Pinowska et al., 1981; Beyerbach et al., 1987; Orłowski et al., 2010, 2012). These birds acquire organochlorine and heavy metal residues through their food items and ingested soil (Hui, 2004; Carpene et al., 2006; Roodbergen et al., 2008; Hiller and Barclay, 2011; Schipper et al., 2012; Fritsch et al., 2012).

The Rook is a long-lived, colonial, omnivorous corvid species, broadly distributed across Eurasia, where its breeding is strongly dependent on agricultural activities (Cramp, 1998). During the breeding period, adult Rooks feed their nestlings with soil invertebrates such as ground-dwelling Coleoptera, earthworms, and plant food such as cereal grain from cultivated crop fields and grasslands (Cramp, 1998; Kasprzykowski, 2003, 2007; Orłowski et al., 2009). These food items may transmit high doses of pesticides and heavy metals from agrochemicals (Malmberg, 1973; Pinowski et al., 1983; Beyerbach et al., 1987; Purchart and Kula, 2007). Recently, we found that Rook eggshells contained the highest concentrations of arsenic (most probably of agricultural origin) ever reported for any biological material, including birds' eggs and the tissues of terrestrial and aquatic animals (Orłowski et al., 2010). Furthermore, we reported that nestling Rooks have cadmium tissue levels suggestive of acute contamination, and also elevated levels of lead (Orłowski et al., 2012). In view of the exceptionally high input and load of agriculturally-related contaminants in the breeding and feeding habitats of Rooks, there is an urgent need for further data on the occurrence of other trace elements, such as lead and cadmium, in this species. We anticipate that the concentrations of these metals in eggshells will be high.

In recent years, the use of birds' eggs and eggshells as indicators of bioaccumulation of environmental pollutants (e.g. non-essential elements and toxic chemicals) has increased, and a large number of papers have been written on this topic (e.g. Burger, 1994, 2002; Furness, 1993; Nyholm, 1998; Eens et al., 2013). However, most studies to date relate to water birds and/or are limited to a relatively small geographical area. Analogous studies of terrestrial species, including those living in agricultural/arable landscapes and/or along habitat gradients from urban to rural, or from less to more intensive agriculture, and/or along a geographical gradient are still scarce (cf. Van den Steen et al., 2009; Eens et al., 2013; Ruuskanen et al., 2014).

Female birds sequester the surplus of non-essential elements in their eggs during egg formation, both in the contents and the shells (Ohlendorf and Harrison, 1986; Burger, 1994, 2002). The transfer rate of non-essential elements into the egg is thought to be related to the concentrations of these elements in the mother's tissues and, ultimately, to the extent of pollution in her environment (Nyholm, 1998). Laboratory experiments with Mallards *Anas platyrhynchos* showed that cadmium concentrations in eggs were low, regardless of the amount consumed by laying females (White and Finley, 1978). Lead and cadmium have been found at baseline levels even in the eggs of birds exposed to these elements (Furness, 1993; Spahn and Sherry, 1999). Some resident passerines feed on food items collected within a limited home range (compared with seabirds and raptors), so the heavy-metal content in those birds, and presumably their eggs, is derived from local sources and can be used to identify local pollution in the foraging area (Dmowski, 1999; Burger, 1994; Furness, 1993). However, in terrestrial birds, some non-essential elements, such as arsenic, copper, lead and nickel, are sequestered primarily into the eggshells rather than into the egg content (cf. Dauwe et al., 1999; Mora, 2003). The eggshell concentration of lead and cadmium is positively correlated with the liver cadmium level and the levels of both these elements in feathers (Dauwe et al., 2005). The eggshells of some terrestrial birds are suitable indicators for lead and cadmium, and the levels of which were higher in eggshells from breeding sites located in industrial areas (Dauwe et al., 1999, 2005). It is worth underlining that, in recent years, the populations of several species of birds that inhabit agricultural areas (including the Rook in Poland) have shown severe declines, an effect of agricultural intensification (PECBMS, 2007; Chodkiewicz et al., 2013). Because pollution from human-related chemicals, including metals, is indicated as a potential cause of some of these population declines in birds, assessing contaminants in diverse feeding guilds of birds along rural–urban gradients is crucial (Kekkonen et al., 2012; Eens et al., 2013).

Here, we examine the levels of four trace elements with potential ecotoxicological effects in eggshells from Rooks breeding in 43 rookeries situated in lowland agricultural areas of central Europe (Poland). Two of these elements are essential [chromium (Cr) and nickel (Ni)] and two are non-essential [cadmium (Cd) and lead (Pb)]. We hypothesised that the concentrations of eggshell elements would differ among rookeries in rural and urban areas, and with respect to geographical location (western versus eastern Poland). The geographical division of rookeries into 'west' and 'east' mirror the levels of agricultural production in the two parts of Poland (see below), with agriculture being more intensive in western Poland and more extensive in the east. We expected to find differences in the levels of elements in eggshells from rural versus urban areas because Rooks forage in the arable land around their breeding colonies (within a radius of ca 3 km from the rookeries; Kasprzykowski, 2003). Furthermore, because western Poland is highly industrialised and has numerous sources of contamination (metallurgical plants, lignite and copper mines, e.g. at Wodzisław Śląski, Głogów), we expected to find higher levels of the target eggshell elements in urban areas.

2. Material and methods

2.1. Study area and breeding colonies

The analytical methods we used, including chemical analysis and classification of rookeries [i.e. west (= intensive agriculture) versus east (= extensive agriculture); and urban vs rural] are described in our earlier work on concentrations of Cu, Zn and As in Rook eggshells (Orłowski et al., 2010). The eggshells we used (in both this and our earlier work) were collected in the spring of 2005 in 43 rookeries, ranging in size from 5 to 480 nests, across Poland (Fig. 1). We picked up the shells (at least two shell fragments from two different eggs) from the ground beneath the nests.

We collected all the samples in the same manner, including those from different geographical locations, and with varying intensities of agricultural production and habitat types. We categorised the results of our analyses according to colony location in the west (intensive agriculture) or east (extensive agriculture) of Poland, as well as according to their location in urban or rural sites. The river Vistula (Wisła) was the major geographic feature used to divide Poland into its eastern and western parts, as it flows through the middle of the country (Fig. 1). We followed Chylarecki et al. (2006) in dividing the intensity of agriculture in Poland into two regions: intensive agriculture (western Poland) and extensive agriculture (eastern Poland).

We sampled eggshells from rookeries located in three different settlement types varying in density of buildings and human population: villages, small towns and large cities. Rookeries from large cities (hereafter 'urban rookeries'; $n = 14$) and rural areas (villages + small towns, hereafter 'rural rookeries'; $n = 29$) were defined on the basis of the number of human inhabitants in each locality, with a population of 50 000 taken to be the threshold for defining a city (after Orłowski and Czapulak, 2007). Furthermore, in some analyses we present data separately for small towns and villages.

Based on 216 monitoring stations (12–20 individual top soil samples [to 20 cm deep] collected in 2005 from one monitoring station)

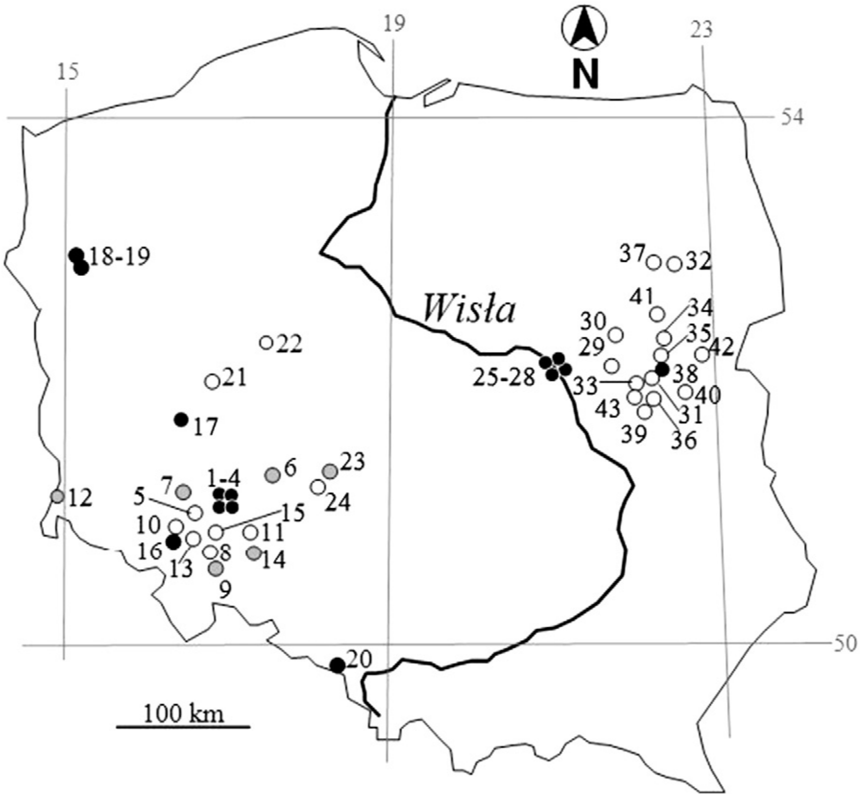

Fig. 1. Distribution of breeding colonies of the Rook *Corvus frugilegus* in villages (○), small towns (●) and large cities (●) in Poland, where eggshell levels of chromium, nickel, cadmium and lead were surveyed. The rookeries are numbered as in Appendix 1. Here, the River Wisła (Vistula) constitutes the border between western and eastern Poland (= intensive vs extensive agriculture; see Material and methods for more details).

distributed across various parts of Poland, the concentration of Cr ranged between 2.1 and 45.5 ppm (average = 11.2 Cr ppm), that of Ni between 1.7 and 83.8 (average = 9.5 Ni ppm), that of Cd between 0.04 and 81.2 ppm (average = 0.69 Cd ppm) and that of Pb between 2.8 and 1033.4 (average = 24.0 Pb ppm) (Terelak et al., 2008). Some breeding colonies included in the present study were located in industrial areas with intensive metallurgy/smelting/mining activities (e.g. the rookeries in Głogów, Wodzisław Śląski) or in large cities (e.g. Wrocław) (Appendix 1; Fig. 1), where elevated soil levels of Ni (up to 17 Ni ppm), Cr (up to 96.3 Cr ppm) and Pb (up to 133 Pb ppm) were found (Meinhardt et al., 2009; Siebielec et al., 2012). In general, however, the concentrations of these elements in top soil from agricultural (rural) areas were lower than the mean values for the whole country given above (Terelak et al., 2008).

2.2. Chemical analysis

Because we were only able to collect a small number of shells at some rookeries, we determined the metal concentration for each colony using just two different shells or their fragments. We used large pieces of shells to ensure that the samples came from different eggs. We removed membrane remnants and visible external dirt from the eggshells before storing them in glass containers for subsequent heavy metal analysis. Three of the co-authors of this paper (WD, PP, RP) performed the chemical analysis at the Department of Aquaculture of the Wrocław University of Environmental and Life Sciences. Prior to chemical analysis, all the eggshells were rinsed twice with water containing detergent and then air-dried. They were then mineralised in a mixture of nitric and perchloric acid in a high-pressure microwave digestion system (MARS-5; CEM, Matthews, NC). We used flame atomic absorption spectroscopy (SpectrAA FS220; Varian, Palo Alto, CA) to determine the metal content. The measurement process was validated using reference material, DORM-3 (fish protein), provided by the National Research Council of Canada Institute for National Measurement. The certified average (± SD) values of element concentrations in the reference material were 1.89 (± 0.17) Cr ppm, 1.28 (± 0.24) Ni ppm, 0.290 (± 0.020) Cd ppm and 0.395 (± 0.050) Pb ppm; in comparison, the average obtained values from the certificate material (six measurements on 0.9-g samples) were 1.97 (± 0.09) Cr ppm, 1.33 (± 0.12) Ni ppm, 0.280 (± 0.013) Cd ppm and 0.381 (± 0.043) Pb ppm. The precision of the method, i.e. the difference between the mean value obtained by analysing a certified reference material and its certified value, performed on the same sample did not exceed 5%. All concentrations of metals were expressed in milligrammes per kilogramme (mg kg^{-1} or parts per million; ppm) of dry mass (d.w.) accurate to two decimal places. The data are presented as arithmetic means with a 95% confidence interval (CI).

2.3. Statistical analyses

The statistical analyses were done using Statistica ver. 7.0 (StatSoft, 2006) and Excel software. The statistical significance level was 0.05.

We tested the differences between heavy metal concentrations of specified data sets for a predefined habitat type (i.e. among villages, small towns and large/industrial cities; and between rural and urban areas), including the combined effect of geographic and habitat division, using ANOVA in General Linear Model (GLM) analysis (StatSoft, 2006), with two (log-transformed: Ni, Cd and Pb) metal concentrations for each rookery. Owing to the small sample size from small towns and large/industrial cities in eastern Poland, we were unable to draw comparisons between the eastern and western regions of the country for these two settlement types; we performed such comparisons only for rookeries located in villages.

Table 1
Comparison (average with 95% c.l.) of the concentrations (ppm) of chromium, nickel, cadmium and lead in Rook *Corvus frugilegus* eggshells from breeding colonies ($N = 43$) in Poland according to their geographical location (=intensity of agriculture): intensive agriculture (west) vs extensive agriculture (east) and habitat division: rural areas (=villages and small towns, <50 000 inhabitants) vs urban areas (=large and industrial cities, >50 000 inhabitants); the same sign (# or $) shows statistically significant differences between colonies according to their geographical and habitat division (see Results section for more statistical details, and Appendix 1 for the colony locations).

Metal	All rookeries ($n = 86$)	Geographical location		Habitat	
		West ($n = 56$)	East ($n = 30$)	Rural area ($n = 58$)	Urban area ($n = 28$)
Cr	6.89 (6.68–7.10)	7.14 (6.85–7.43)#	6.42 (6.22–6.61)#	6.57 (6.34–6.79)$	7.55 (7.18–7.92)$
Ni	1.79 (1.68–1.90)	1.84 (1.67–2.00)	1.71 (1.63–1.79)	1.68 (1.57–1.79)#	2.02 (1.79–2.26)#
Cd	0.51 (0.49–0.53)	0.51 (0.48–0.54)	0.50 (0.48–0.52)	0.50 (0.48–0.52)	0.52 (0.47–0.57)
Pb	3.29 (2.91–3.67)	3.45 (2.86–4.03)	3.00 (2.86–3.15)	2.90 (2.69–3.11)#	4.11 (3.03–5.18)#

3. Results

Overall, the concentration of the target elements in Rook eggshells from all rookeries in Poland varied over quite small ranges: the difference between extreme values was 1.8-fold (range of concentrations = 5.21–9.40 Cr ppm) for Cr, 3.5-fold (1.15–4.07 Ni ppm) for Ni, 2.6-fold (0.34–0.91 Cd ppm) for Cd, and was the highest – 6.7-fold (1.71–11.53 Pb ppm) – in the case of Pb (Appendix 1). Interestingly, eggshells from two rookeries from western Poland (Fig. 1: colony nos. 20 and 21) had the highest concentrations of three elements: Cr, Ni and Cd. Similarly, another colony from the large city, Wrocław (633 000 inhabitants; Fig. 1: no. 1), had the highest concentrations of Ni, Cd and Pb (Appendix 1). In five (12%) rookeries, the level of Cr was >8 ppm, and in two (5%) rookeries, the Ni level was >3 ppm. A Cd level ≥0.6 ppm was found in three (7%) rookeries. Pb concentrations were the most diverse: in 40 (93%) rookeries they were <5 Pb ppm; only in three of them were its concentrations higher (Appendix 1).

Rookeries situated in western Poland had significantly higher concentrations of Cr (GLM: $F_{1,84} = 11.78$, $p = 0.001$) (Table 1), but concentrations of the other three elements did not differ between regions (all $p \geq 0.260$) (Table 1). The higher Cr eggshell level in western Poland resulted from a disproportionately high proportion of urban rookeries in the sample from that area (Fig. 1): when only rural rookeries were taken into consideration, the Cr concentration did not differ between western and eastern Poland (GLM, $F_{1,44} = 2.83$, $p = 0.100$). Similarly, the concentration of the three other eggshell elements did not differ between villages from western and eastern Poland (all $p \geq 0.342$).

Across sites, eggshells from urban rookeries had significantly higher levels of Cr (GLM: $F_{1,84} = 23.24$, $p < 0.0001$), Ni ($F_{1,84} = 9.08$, $p = 0.003$) and Pb ($F_{1,84} = 9.55$, $p = 0.003$) than eggshells from rural areas (Table 1). The analysis of combined geographical × habitat effect did not show any significant interaction: Cr ($p = 0.100$), Ni ($p = 0.417$), Cd ($p = 0.430$) and Pb ($p = 0.455$).

Further GLM analysis with the three types of settlement (villages, small towns and large/industrial cities) confirmed (as did the previous analysis) the significant differences in concentrations of Cr ($F_{2,83} = 11.64$, $p < 0.0001$), Ni ($F_{2,83} = 5.54$, $p = 0.005$) and Pb ($F_{2,83} = 4.14$, $p = 0.019$) among settlement types (Fig. 2). Post-hoc analysis showed that eggshell concentrations of Cr, Ni and Pb were the highest in colonies located in large, industrial cities, where ambient levels of these elements were significantly higher than in small towns and villages. Concentrations of eggshell elements did not differ between small towns and villages (Fig. 2).

4. Discussion

We found high levels of four non-essential elements in Rook eggshells in Poland. However, with the exception of the high Cr concentration, these levels were within the ranges reported in earlier papers.

4.1. Eggshell metal levels: comparison and variability in various bird species

Across a range of bird species, eggshell cadmium concentrations are low: <0.3 Cd ppm (cf. Hashmi et al., 2013). However, cadmium (Cd) concentrations in whole eggs are higher: among various raptors, seabirds and fish-eating birds, concentrations ranged from 0.02 to 6 Cd ppm (Burger, 2002). Empirical evidence consistently endorses the pattern

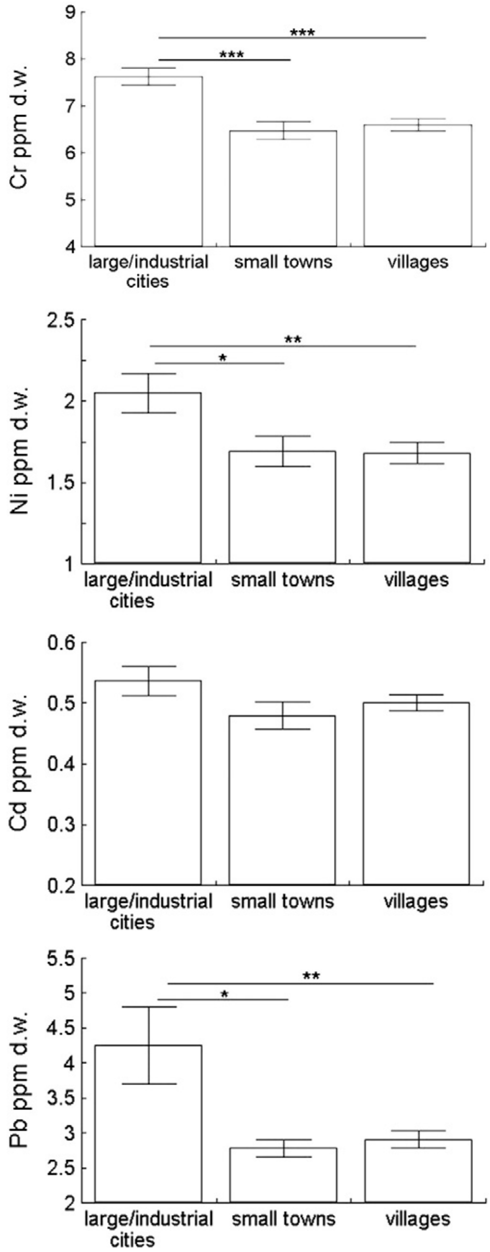

Fig. 2. Comparison of average (±1 SE) concentrations (ppm) of chromium, nickel, cadmium and lead in Rook *Corvus frugilegus* eggshells from breeding colonies situated in large/industrial cities, small towns and villages in Poland (see Appendix 1 for the habitat division of the rookeries); statistically significant differences obtained in the post-hoc comparison are indicated: *$p \leq 0.05$, **$p < 0.01$, and ***$p < 0.001$.

that Cd concentrations differ between eggshells and egg contents. For example, Burger (1994) found that Cd concentrations were 5 times higher in Herring Gull *Larus argentatus* eggshells than in the egg contents: 0.05 vs 0.01 Cd ppm. In Roseate Terns *Sterna dougallii*, the relationship was reversed, with eggshells having a lower Cd concentration than the egg contents: 0.1 vs 0.2 Cd ppm. In Great Tits *Parus major* from a reference site, eggshells had higher Cd levels than the egg contents, whilst at a polluted site, the opposite was true (Dauwe et al., 1999).

Our recent work showed that the Cd concentration in nestling Rooks, including early-hatched ones (1–2 days old, including nestlings without ingested food) from several rookeries in north-eastern Poland (also included in the present study on eggshell metal levels) was equally high in their tissues (liver, kidney, lung, muscles and bones: range = 15.52–18.8 Cd ppm d.w.) (Orłowski et al., 2012). However, the level of this metal in kidneys was positively correlated with the number of animal food items (primarily various ground-dwelling Coleoptera and their larvae) in their stomach content (Orłowski et al., 2013). In view of the average eggshell Cd level found in this study – 0.51 Cd ppm (with a relatively small range: 0.49–0.53 Cd ppm) – it is evident that in the Polish Rook population examined here this metal (reported at an apparently elevated level) is sequestered primarily into the egg contents. This may indirectly suggest the maternal transfer of this element into the eggs through the female's bloodstream (Guirlet et al., 2008).

The mean levels of Cr, Cd and Pb in eggshells of Herring Gull and Roseate Tern from the coastal area of Long Island, on the Atlantic coast of the north-eastern United States, were 1.6/1.2, 0.05/0.1 and 0.3/1.2 for both these species respectively (Burger, 1994). In general, considering only the lower value from abovementioned study, these concentrations were 5–11 times lower than in our study. Kraus (1989) reported a relatively high concentration of 1.8 Cd ppm in eggshells of Tree Swallows *Tachycineta bicolor* from an area polluted by effluent from several sewage treatment plants and large landfill sites. Recently, Hashmi et al. (2013) found that Cd levels ranged between 0 and 2.8 Cd ppm in eggshells of Cattle Egrets *Bubulcus ibis* and Little Egrets *Egretta garzetta* from eastern Pakistan; the latter value is one of the highest Cd levels ever recorded in eggshells.

Chromium is an essential nutrient for animals that can play an important beneficial role in interaction with some toxic elements, although at higher concentrations, Cr is mutagenic, carcinogenic and teratogenic to a wide variety of organisms (Eisler, 1986). Its concentrations in non-biological materials are elevated in the vicinity of industrial operations, municipal waste treatment facilities, including sludge from animal husbandry, hence may have serious implications for wildlife when the sludge is applied to croplands (Eisler, 1986). Compared to the published data on the level of Cr in avian eggs, that is, whole homogenised eggs, egg content and eggshells (cf. Ikemoto et al., 2005; Custer et al., 2006; Hashmi et al., 2013; Mora, 2003; Dauwe et al., 2005; Al-Obaidi et al., 2012), the average level of this metal in our study – 6.89 (range = 5.21–9.40) Cr ppm – should be regarded as decidedly high. Furthermore, the concentration >4 Cr ppm d.w. in bird tissues should be treated as presumptive evidence of contamination by this metal, although its concentration in various biological materials varies widely (Eisler, 1986). The Cr concentration in whole eggs of several raptors, seabirds and some fish-eating birds ranged between 0.01 and 10 Cr ppm wet weight (≈0.02–24 Cr ppm d.w.) (Burger, 2002). However, the concentration of 3.09 mg Cr d.w. already found in whole eggs of Franklin's Gulls *Larus pipixcan* was defined as extremely high (Burger and Gochfeld, 1996, but see the comment in Custer et al., 2006). Overall, in birds, the Cr concentration is higher in the egg content than in the shells. For instance, an average of 1.6 and 1.2 Cr ppm was found in the eggshells of Herring Gulls and Roseate Terns, compared to 2.7 and 2.8 ppm Cr in the egg contents (Burger, 1994). An exceptionally high eggshell Cr level (average for three eggshells examined: 173.6 Cr ppm) along with equally high levels of Pb (90.9 Pb ppm) and Ni were found in Tree Swallows feeding on aerial insects developed in polluted wetland sediments in an estuarine habitat in New Jersey (Kraus, 1989); here it is worth pointing out that both these values are most likely the highest reported Cr and Pb concentrations reported in avian eggshells. More recently, the average eggshell Cr levels in four sedentary species – House Sparrow *Passer domesticus*, White-eared Bulbul *Pycnonotus leucotis*, Collared Dove *Streptopelia decaocto* and Rock Dove *Columba livia* – from urban areas of Baghdad ranged from 2.33 to 3.76 Cr ppm (Al-Obaidi et al., 2012).

Data on Ni eggshell concentrations in birds are quite limited, but previous studies have shown that this element is concentrated primarily in the shells rather than in the egg content (Mora, 2003; Dauwe et al., 2005). In areas contaminated by Ni, concentrations of this metal were elevated in feathers, eggs and internal tissues of birds when compared to conspecifics collected at reference sites (Eisler, 1998). In two small passerines, Yellow-breasted Chat *Icteria virens* and Willow Flycatcher *Empidonax traillii extimus*, from Arizona, Mora (2003) found that Ni concentrations were respectively 16 and 26 times greater in the eggshells (average = 4.1 and 6.5 ppm Ni) than in the egg contents (<0.5) in both these species. A considerably higher Ni concentration was found in the eggs of Great Tits breeding in close proximity to a heavy metal smelter: up to an average of 10.8 (max = 13.4) ppm Ni d.w. in eggshells versus 0.14 (max = 1) ppm Ni d.w. in the egg contents (Dauwe et al., 2005). Exceptionally high Ni concentrations were reported more recently in the eggshells of Pied Flycatcher *Ficedula hypoleuca*. In 15 different populations of this species from various parts of Europe, the eggshell concentration of this metal ranged between 23.2 and 43.5 Ni ppm (Ruuskanen et al., 2014). To the best of our knowledge, these values are probably the highest Ni concentrations in avian eggshells yet reported. This is very interesting, because the levels of many other elements in that study (including some toxic ones like Pb) were very low (cf. Ruuskanen et al., 2014).

Generally, Pb (trialkylleads and dialkylleads) rapidly cross biological membranes in bird eggs and accumulate in the yolk and developing embryo (Forsyth et al., 1985). However, data on the primary location of Pb sequestration into bird eggs (eggshell or egg content) are inconclusive. In Herring Gulls and Roseate Terns, the average Pb concentration was significantly higher (approximately 2–8 times) in the egg content than in the eggshells: 2.5 vs 0.3 Pb ppm and 2.3 vs 1.2 Pb ppm, for these species respectively (Burger, 1994). Conversely, Kraus (1989; analysing eggs from a heavily polluted area), Dauwe et al. (1999, 2005; eggs from contaminated and reference sites), Mora (2003; eggs with a low Pb content) and Swaileh and Sansur (2006; eggs from urban and rural sites varying in Pb levels) found that Pb levels were significantly higher in eggshells than in egg contents. In spite of these discrepancies, considering earlier comparative studies of Pb eggshell levels in birds from contaminated versus reference sites, our findings suggest a relatively high level of this metal, which is indicative of the high bioaccumulation of Pb in the Rook eggshells, a matter of environmental concern (cf. Dauwe et al., 1999, 2005; Mora, 2003; Ayas, 2007; Hashmi et al., 2013). Kraus (1989) found exceptionally high Pb levels in Tree Swallow eggshells (90.9 Pb ppm) and at least 2.3 Pb ppm in Tree Swallow embryos. Dauwe et al. (1999) reported the following levels in Great and Blue Tit eggshells from polluted sites: 15 Pb ppm, compared to 0.37 Pb ppm at reference sites. Similarly, Rodriguez-Navarro et al. (2002) found (average) 0.37 (range = 0.08–2.62) Pb ppm, in comparison to 0.23 (0.10–0.46) Pb ppm at a reference site in eggshells of Clapper Rails *Rallus longirostris* from a contaminated salt marsh in coastal Georgia. House Sparrow *P. domesticus* eggshells from urban and rural areas of Palestine contained a relatively high Pb level: 3.3 Pb ppm (range = 1.5–4.8). Finally, the above comparison with other results indicates an overall high bioaccumulation of Pb in Rook eggshells in the whole study population in Poland.

4.2. Effect of habitat and rookery location on eggshell metal levels

Despite the small number of shells collected in individual rookeries our findings seem to suggest an effect of industry and/or urbanisation on the concentrations of the target eggshell elements. This may be so, as in the case of Pb, the highest level (>11 ppm Pb) of which was found in the industrial city of Głogów (Fig. 1: no. 17), where a large non-ferrous (copper) metallurgical plant is located. Furthermore, our findings seem to suggest a relatively large variability in the level of the toxic metals Cd and Pb in eggshells from neighbouring colonies in large cities, such as Wrocław or Warsaw (cf. Appendix 1). On the other hand, if we consider the entire data set of eggshells from urban/rural rookeries, our study to a greater extent reflects overall inter-habitat differences than intra-colony variability in target element levels. An important result of our study, are the significantly higher levels of Cr, Ni and Pb in Rook eggshells from urban areas, primarily from large/industrial cities, than in eggshells from rural areas. The lack of significant differences in the concentrations of the target elements between small towns and villages (cf. Fig. 2) from western and eastern Poland did not support our initial prediction that metal concentrations would be higher in areas with more intensive agriculture as a result of the intensive application of organic/mineral fertilisers across the whole of Poland (Dach and Starmans, 2005), probably reflecting a uniform level of these elements in the invertebrates that Rooks consume. For instance, in both western and eastern Poland, including the vicinities of Wrocław and Siedlce, where a large numbers of the rookeries were situated (Fig. 1), concentrations of the four target elements were similar and did not exceed 15.9 Cr ppm, 11.6 Ni ppm, 0.28 Cd ppm, and 9.0 Pb ppm (Terelak et al., 2008).

In urban areas, on the other hand, the overall concentrations of these four elements in the soil are related to anthropogenic activities. Thus, mining and smelting are responsible for elevated soil Cr, Ni, Cd and Pb levels; disposal of animal faeces used as organic fertilisers (slurry and manure) and the application of agrochemicals and pesticides give rise to Cr, Pb and Cd residues; and combustion of fuels, including petrol, increases Pb levels (Eisler, 1986, 1998; Burger, 2008). Our findings that Cr, Ni and Pb concentrations are higher in Rook eggshells from urban rookeries are consistent with a link to anthropogenic pollution; populations of other wild birds demonstrate a similar gradient of pollution from rural to urban areas (Scheifler et al., 2006; Roux and Marra, 2007; Bichet et al., 2013), and pollutants appear to enter birds' bodies through their diet (Roodbergen et al., 2008; Fritsch et al., 2012; Orłowski et al., 2013). The higher content of some metals in urban soils in comparison to suburban or rural sites determines the level of contaminants in prey consumed (Scheifler et al., 2006; Roux and Marra, 2007; Fritsch et al., 2012). For instance, the soil Pb content in urban and suburban gardens is on average nearly nine times higher than that in rural gardens, i.e. 275 Pb ppm compared to 25 Pb ppm (Roux and Marra, 2007). Similarly, earthworms from urban sites had significantly higher Pb concentrations than earthworms from rural sites (more than twice as high, depending also upon the type of earthworm community) (Scheifler et al., 2006). Finally, it seems that our findings are in line with the most recent study on Pied Flycatchers, which shows a significant positive relationship between eggshell Pb concentrations and soil Pb levels; this suggests rapid bioaccumulation of this toxic element in females during egg-laying at the breeding site (Ruuskanen et al., 2014).

The eggshell levels of Cr, Ni, and Pb we found most likely reflect local sources of contamination, since they varied along a pollution gradient. The presence of heavy industry (e.g. a metallurgical plant or mining activity) or intensive urbanisation (e.g. the presence of busy roads or railway lines in large cities) may affect eggshell metal levels if adult Rooks forage in these polluted areas during the breeding period, as our data indicate (authors' unpubl. data). The lack of a habitat effect (urban versus rural) on eggshell Cd concentrations is most likely an effect of the low permeability of the calcified eggshell to Cd; this element occurs at baseline levels even in the eggs of birds exposed to Cd (Spahn and Sherry, 1999). Our findings suggest indirectly that, in female Rooks, bioaccumulation of Cr, Ni and Pb proceeds intensively at the time of egg production (e.g. Roodbergen et al., 2008), whereas Cd is accumulated over a longer period. Alternatively, a physiological mechanism may limit Cd sequestration into eggshells since, as we discussed above, this metal is very probably transferred more effectively into the egg contents.

5. Concluding remarks

The Rook population of central Europe shows high concentrations of some trace metals from industry and the residues of agricultural pesticides (Malmberg, 1973; Pinowska et al., 1981; Pinowski et al., 1983; Beyerbach et al., 1987; Orłowski et al., 2010, 2012). Rook eggshells are useful biological indicators of local exposure to Cr, Ni and Pb. These three trace metals showed site-related differences in concentrations, with higher concentrations in eggshells from breeding colonies in urban or industrial areas than in rural areas. The overall high bioaccumulation of all four elements in Rooks appeared to occur along the dietary pathway resulting from the pollution gradient of soil: villages and small towns exhibited equivalent levels of these metals, whereas in large cities these levels were higher.

Because Rooks spend a long period of time on their wintering grounds (in western Europe; Cramp, 1998; Gromadzki and Mokwa, 2005), the accumulation of Cr, Ni and Pb (and Cu, the concentration of which was also significantly higher at urban rookeries; Orłowski et al., 2010) in eggs must occur during just a few days, when the females are already at their breeding sites. Earlier work demonstrated that passerines acquire most nutrients immediately prior to and during egg laying, resulting in the rapid trophic transfer of nutrients and contaminants to their eggs; the level of contaminants in eggs thus reflects the recent diet (cf. Morrissey et al., 2010; Ruuskanen et al., 2014). Our study suggests that female Rooks vary in the intensity of trace metal bioaccumulation in their eggshells. Bioaccumulation of Cu, Cr, Ni and Pb appears to be site-dependent. On the other hand, eggshell levels of Cd were relatively high. These are probably regulated physiologically and very likely reflect the level of this element in female tissues. They may also be an indication of a generally severe Cd burden in the soil environment and the invertebrate prey of Rooks (discussed in Pinowski et al., 1983; Orłowski et al., 2012, 2013).

Further ecotoxicological studies of egg physiology and its elemental composition are urgently needed, especially in the context of the dietary uptake of agriculturally and industrial-related compounds. They should address inter alia the effect of laying sequence and assess inter-colony variability on the bioaccumulation of various elements in the contents and shells of eggs in relation to the age of females and their life history traits. Differences in local land-uses around the breeding colonies of Rooks and other soil-invertebrate feeding birds breeding along an urbanisation/industrialisation gradient should also be taken into consideration. The Rook breeding population in Poland has decreased by 40% over the last 10 years — 2002–2012 (GIOŚ, 2013). Ecotoxicological studies of declining species living in environments with a high input of anthropogenic contaminants are of critical importance for identifying the reasons for the decrease in numbers or disappearance of species (cf. Kekkonen et al., 2012).

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We are grateful to W. Bena, P.T. Dolata, S. Jędrzejewski, K. Kujawa, R. Kruszyk, S. Kuźniak, M and U. Martini, R. Piekarski, J. Pietrowiak, M. Rachel, J. Ratajczak, M. Słupek, A. Sokołowska, T. Stawarczyk, P. Tryjanowski, T. Wilżak, T. Zarzycki and the Management of the Landscape Parks in Sieradz for participating in the ecotoxicological study of the Rook in Poland. We appreciate the improvements in the English usage made by Caitlin Stern through the Association of Field Ornithologists' programme of editorial assistance, and by Peter Senn.

Appendix 1

Concentration (ppm) of chromium, nickel, cadmium and lead in eggshells of the Rook *Corvus frugilegus* from 43 breeding colonies in Poland. Metal levels in two shells per colony are shown. [1]Number of the rookery, as in Fig. 1. [2]Status of the colony: rural rookeries = villages (n = 23 colonies) + small towns (<50 000 inhabitants; n = 6); large/industrial city (>50 000 inhabitants; n = 14). Three highest concentrations are marked in bold.

No[1]	Location	Habitat[2]	Status of colony		Concentration (ppm)							
			Rural (R)/urban (U)	West (W)/east (E)	Cr		Ni		Cd		Pb	
1	Wrocław (1)	Large city	U	W	7.07	7.12	**2.32**	**2.33**	**0.60**	**0.60**	**9.43**	**9.44**
2	Wrocław (2)	Large city	U	W	6.06	6.09	1.77	1.79	0.53	0.54	2.77	2.77
3	Wrocław (3)	Large city	U	W	7.20	7.24	1.94	1.95	0.51	0.52	2.47	2.47
4	Wrocław (4)	Large city	U	W	7.65	7.70	1.73	1.74	0.43	0.43	1.72	1.72
5	Pietrzykowice	Village	R	W	7.04	7.10	2.05	2.06	0.57	0.57	2.21	2.21
6	Oleśnica	Small town	R	W	6.98	7.04	2.13	2.14	0.59	0.59	3.01	3.01
7	Środa Śląska	Small town	R	W	6.45	6.49	2.03	2.04	0.57	0.58	3.02	3.02
8	Przerzeczyn Zdrój	Village	R	W	6.13	6.18	1.50	1.51	0.47	0.48	2.28	2.29
9	Ząbkowice Śląskie	Small town	R	W	7.39	7.45	1.94	1.95	0.51	0.52	3.09	3.11
10	Pszenno	Village	R	W	6.10	6.13	1.15	1.16	0.37	0.37	2.31	2.33
11	Borek Strzeliński	Village	R	W	6.83	6.87	1.38	1.38	0.49	0.49	2.35	2.36
12	Zgorzelec	Small town	R	W	5.66	5.71	1.57	1.57	0.46	0.46	3.37	3.39
13	Zebrzydów	Village	R	W	8.48	8.49	1.26	1.26	0.47	0.47	**6.37**	**6.39**
14	Strzelin	Small town	R	W	6.79	6.79	1.21	1.21	0.40	0.40	2.07	2.08
15	Łagiewniki	Village	R	W	7.40	7.41	1.36	1.36	0.43	0.43	2.56	2.57
16	Świdnica	Large city	U	W	6.58	6.59	1.67	1.68	0.34	0.35	2.27	2.29
17	Głogów	Large city/industry	U	W	7.36	7.37	1.89	1.90	0.52	0.52	**11.30**	**11.53**
18	Gorzów Wlkp (1)	Large city	U	W	**9.20**	**9.20**	1.49	1.50	0.40	0.40	2.13	2.14
19	Gorzów Wlkp (2)	Large city	U	W	7.41	7.42	1.88	1.89	0.51	0.51	3.64	3.66
20	Wodzisław Śl.	Large city/industry	U	W	**9.34**	**9.41**	**4.05**	**4.07**	**0.90**	**0.91**	3.95	3.95
21	Gołębin Stary	Village	R	W	**8.87**	**8.93**	**3.31**	**3.32**	**0.84**	**0.84**	2.42	2.42
22	Żerków	Village	R	W	5.22	5.26	1.27	1.28	0.43	0.43	2.26	2.27
23	Kępno	Small town	R	W	5.33	5.38	1.27	1.28	0.47	0.47	2.59	2.61
24	Laski	Village	R	W	5.56	5.61	1.46	1.46	0.45	0.46	2.37	2.39
25	Warszawa (1)	Large city	U	W	8.36	8.36	1.88	1.89	0.47	0.47	4.75	4.76
26	Warszawa (2)	Large city	U	W	7.38	7.39	1.90	1.91	0.45	0.45	3.26	3.28
27	Warszawa (3)	Large city	U	W	7.86	7.87	1.94	1.95	0.50	0.50	2.94	2.96
28	Warszawa (4)	Large city	U	W	7.82	7.83	2.01	2.02	0.55	0.55	3.35	3.37
29	Podnieśno	Village	R	E	7.09	7.14	1.67	1.68	0.38	0.38	2.18	2.18
30	Wyszków	Village	R	E	7.58	7.60	1.90	1.91	0.54	0.54	3.54	3.54
31	Iganie	Village	R	E	6.40	6.45	1.97	1.98	0.56	0.56	3.49	3.49
32	Biodry	Village	R	E	6.35	6.40	1.87	1.88	0.40	0.40	2.54	2.54
33	Żelków	Village	R	E	7.18	7.21	1.42	1.43	0.50	0.50	2.57	2.59
34	Czyżew	Village	R	E	6.06	6.09	1.53	1.54	0.47	0.47	2.72	2.74
35	Zembrów	Village	R	E	5.78	5.83	1.59	1.60	0.52	0.52	3.13	3.15
36	Stoczek Łukowski	Village	R	E	5.61	5.65	1.55	1.55	0.53	0.53	3.08	3.11
37	Wizna	Village	R	E	6.73	6.78	1.61	1.62	0.52	0.52	3.00	3.02
38	Siedlce	Large city	U	E	6.23	6.28	1.77	1.77	0.58	0.58	3.31	3.33
39	Oleśnica	Village	R	E	6.09	6.14	1.42	1.43	0.53	0.53	2.84	2.86
40	Mokobody	Village	R	E	6.14	6.19	1.64	1.64	0.54	0.55	3.10	3.12
41	Seroczyn	Village	R	E	6.18	6.23	1.93	1.94	0.52	0.52	3.58	3.61
42	Mordy	Village	R	E	6.27	6.32	1.56	1.56	0.50	0.50	2.77	2.79
43	Toczyska	Village	R	E	6.21	6.26	2.15	2.16	0.47	0.48	3.07	3.09

References

Al-Obaidi FA, Mehdi B, Al-Shadeedi S. Identification of inorganic elements in egg shell of some wild birds in Baghdad. Adv Appl Sci Res 2012;3:1454–8.

Ayas Z. Trace element residues in eggshells of grey heron (*Ardea cinerea*) and black-crowned night heron (*Nycticorax nycticorax*) from Nallihan Bird Paradise, Ankara-Turkey. Ecotoxicology 2007;16:1573–3017.

Beyerbach M, Büthe A, Heidmann WA, Dettmer R, Knüwer H. Chlorierte Kohlenwasserstoffe in Eiern und Lebern von Saatkrähen (*Corvus frugilegus*) aus niedersächsichen Brutkolonien. J Ornithol 1987;128:277–90.

Bichet C, Scheifler R, Coeurdassier M, Julliard R, Sorci G, Loiseau C. Urbanization, trace metal pollution, and malaria prevalence in the house sparrow. PLoS ONE 2013;8:e53866. http://dx.doi.org/10.1371/journal.pone.0053866.

Burger J. Heavy metals in avian eggshells: another excretion method. J Toxicol Environ Health 1994;41:207–20.

Burger J. Food chain differences affect heavy metals in bird eggs in Barnegat Bay, New Jersey. Environ Res 2002;90:33–9.

Burger J. Assessment and management of risk to wildlife from cadmium. Sci Total Environ 2008;389:37–45.

Burger J, Gochfeld M. Heavy metal and selenium levels in Franklin's gull (*Larus pipixcan*) parents and their eggs. Arch Environ Contam Toxicol 1996;30:487–91.

Carpene E, Andreani G, Monari M, Castellani G, Isani G. Distribution of Cd, Zn, Cu and Fe among selected tissues of the earthworm (*Allobophora caliginosa*) and Eurasian woodcock (*Scolopax rusticola*). Sci Total Environ 2006;363:126–35.

Castilla AM, Dauwe T, Mora I, Malone J, Guitart R. Nitrates and herbicides cause higher mortality than the traditional organic fertilizers on the grain beetle, *Tenebrio molitor*. Bull Environ Contam Toxicol 2010;84:101–5.

Chodkiewicz T, Neubauer G, Chylarecki P, Sikora A, Cenian Z, Ostasiewicz M, et al. Monitoring populacji ptaków Polski w latach 2012–2013. Biuletyn Monitoringu Przyrody 2013;11:1–72.

Chylarecki P, Jawińska D, Kuczyński L. Common breeding bird monitoring in Poland: annual report 2003–2004. Warsaw, Poland: Polish Society for the Protection of Birds; 2006 [30 pp.].

Cramp S, editor. The complete birds of the Western Palearctic on CD-ROM. Oxford: Oxford University Press; 1998.

Custer CM, Custer TW, Warburton D, Hoffman D, Bickham J, Matson C. Trace element concentrations and bioindicator response in tree swallows from northwestern Minnesota. Environ Monit Assess 2006;118:247–66.

Dach J, Starmans D. Heavy metals balance in Polish and Dutch agronomy: actual state and previsions for the future. Agric Ecosyst Environ 2005;107:309–16.

Dauwe T, Bervoets L, Blust R, Pinxten R, Eens M. Are eggshells and egg contents of Great and Blue Tits suitable as indicators of heavy metal pollution? Belg J Zool 1999;129:439–47.

Dauwe T, Janssens E, Bervoets L, Blust R, Eens M. Heavy metal concentrations in female laying great tits (Parus major) and their clutches. Arch Environ Contam Toxicol 2005;49:249–56.

De Vries W, Romkens PF, van Leeuwen T, van Bronswijk JJ. Heavy metals. In: Hagyart PM, Jarvis SC, editors. Agriculture hydrology and water quality. Oxon: CAB Int.; 2002. p. 107–32.

Dmowski K. Birds as bioindicators of heavy metal pollution: review and examples concerning European species. Acta Ornithol 1999;34:1–25.
Dmowski K, Golimowski J. Feathers of the magpie (*Pica pica*) as a bioindicator material for heavy metal pollution assessment. Sci Total Environ 1993;139(140):251–8.
Eens M, Jaspers VLB, Van den Steen E, Bateson M, Carere C, Clergeau P, et al. Can starling eggs be useful as a biomonitoring tool to study organohalogenated contaminants on a worldwide scale? Environ Int 2013;51:141–8.
Eisler R. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report, 85 (1.6). ; 1986. [60 pp.].
Eisler R. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Science Report USGSContaminant Hazard Reviews. Report No. 34; 1998. [95 pp.].
Forsyth DS, Marshall WD, Collette MC. Interaction of alkyllead salts with avian eggs. J Environ Sci Health 1985;20:177–91.
Fritsch C, Coeurdassier M, Faivre B, Baurand P, Giraudoux P, van den Brink N, et al. Influence of landscape composition and diversity on contaminant flux in terrestrial food webs: a case study of trace metal transfer to European blackbirds *Turdus merula*. Sci Total Environ 2012;432:275–87.
Furness RW. Birds as monitors of pollutants. In: Furness RW, Greenswood JJ, editors. Birds as monitors of environmental change. London: Chapman and Hall; 1993. p. 86–141.
GIOŚ. Monitoring ptaków w tym monitoring obszarów specjalnej ochrony ptaków Natura 2000 Faza IV, lata 2012–2015. Warszawa: Główny Inspektorat Ochrony Środowiska; 2013 [221 pp.].
Gromadzki M, Mokwa K. Migrations and wintering of the Rook *Corvus frugilegus* in Poland—analysis based on recoveries. In: Jerzak L, Kavanagh BP, Tryjanowski P, editors. Corvids of Poland. Poznań: Bogucki Wydawnictwo Naukowe; 2005. p. 319–31.
Guirlet E, Das K, Girondot M. Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. Aquat Toxicol 2008;88:267–76.
Hashmi MZ, Malik R, Shahbaz M. Heavy metals in eggshells of cattle egret (*Bubulcus ibis*) and little egret (*Egretta garzetta*) from the Punjab province, Pakistan. Ecotoxicol Environ Saf 2013;89:158–65.
Hiller BJ, Barclay JS. Concentrations of heavy metals in American Woodcock harvested in Connecticut. Arch Environ Contam Toxicol 2011;60:156–64.
Hui CA. Geophagy and potential contaminant exposure for terrestrial vertebrates. Rev Environ Contam Toxicol 2004;183:115–34.
Ikemoto T, Kunito T, Tanabe S, Tsurumi M, Sato F, Oka N. Non-destructive monitoring of trace element levels in short-tailed albatrosses (*Phoebastria albatrus*) and black-footed albatrosses (*Phoebastria nigripes*) from Torishima Island, Japan, using eggs and blood. Mar Pollut Bull 2005;51:889–95.
Kasprzykowski Z. Habitat preferences of foraging Rooks *Corvus frugilegus* during the breeding period in the agricultural landscape of eastern Poland. Acta Ornithol 2003;38:27–31.
Kasprzykowski Z. Reproduction of the rook *Corvus frugilegus* in relation to the colony size and foraging habitats. Folia Zool 2007;56:186–93.
Kekkonen J, Hanski IK, Väisänen RA, Brommer JE. Levels of heavy metals in House Sparrows (*Passer domesticus*) from urban and rural habitats of southern Finland. Ornis Fenn 2012;89:91–8. [2007].
Kraus ML. Bioaccumulation of heavy metals in prefledgling tree swallows, *Tachycineta bicolor*. Bull Environ Contam Toxicol 1989;43:407–14.
Linden A, Andersson K, Oskarsson A. Cadmium in organic conventional pig production. Arch Environ Contam Toxicol 2001;40:425–31.
Malmberg T. Pesticides and the rook *Corvus frugilegus* in Scania, Sweden between 1955 and 1970. Oikos 1973;24:377–87.
Meinhardt B, Danielska I, Kubacka L, Hanula P. Ocena stopnia zanieczyszczenia gleb w województwie dolnośląskim w 2008 roku obszary bezpośrednio zagrożone zanieczyszczeniami. Wojewódzki Inspektorat Ochrony Środowiska we Wrocławiu; 2009.
Mora MA. Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. Environ Pollut 2003;123:393–400.
Morrissey CA, Elliott JE, Ormerod SJ. Diet shifts during egg laying: implications for measuring contaminants in bird eggs. Environ Pollut 2010;158:447–54. [2003].
Nyholm NE. Influence of heavy metals exposure during different phases of the ontogeny on the development of Pied Flycatchers, *Ficedula hypoleuca*, in natural population. Arch Environ Contam Toxicol 1998;35:632–7.
Ohlendorf HM, Harrison CS. Mercury, selenium, cadmium and organochlorines in eggs of three Hawaiian seabird species. Environ Pollut 1986;11:169–91.
Olsson I, Eriksson J, Öborn I, Skerfving S, Oskarsson A. Cadmium in food production systems: a health risk for sensitive population groups. Ambio 2005;34:344–51.
Orłowski G, Czapulak A. Different extinction risks of the breeding colonies of Rook *Corvus frugilegus* in rural and urban areas of SW Poland. Acta Ornithol 2007;42:145–55.
Orłowski G, Kasprzykowski Z, Zawada Z, Kopij G. Stomach content and grit ingestion by Rook *Corvus frugilegus* nestlings. Ornis Fenn 2009;86:117–22.
Orłowski G, Kasprzykowski Z, Dobicki W, Pokorny P, Polechonski R. Geographical and habitat differences in concentrations of copper, zinc and arsenic in eggshells of the Rook *Corvus frugilegus* in Poland. J Ornithol 2010;151:279–86.
Orłowski G, Kamiński P, Kasprzykowski Z, Zawada Z, Koim-Puchowska B, Szady-Grad M, et al. Essential and nonessential elements in nestling Rooks *Corvus frugilegus* from eastern Poland with a special emphasis on their high cadmium contamination. Arch Environ Contam Toxicol 2012;63:601–11.
Orłowski G, Kamiński P, Kasprzykowski Z, Zawada Z. Relationships between stomach content and concentrations of essential and nonessential elements in tissues of omnivorous nestling Rooks *Corvus frugilegus*: Is the size and composition of stomach content relevant? Folia Zool 2013;62:281–8.
PECBMS. State of Europe's common birds. Prague, Czech Republic: CSO/RSPB; 2007 [23 pp.].
Pinowska B, Kraśnicki K, Pinowski J. Estimation of the degree contamination of granivorous birds with heavy metals in agricultural and industrial landscape. Ekol Pol 1981;A 7:137–49.
Pinowski J, Pinowska B, Kraśnicki K, Tomek T. Chemical composition of growth in nestling Rooks *Corvus frugilegus*. Ornis Scand 1983;14:289–98.
Pinowski J, Barkowska M, Kruszewicz AH, Kruszewicz AG. The causes of the mortality of eggs and nestlings of *Passer* spp. J Biosci 1994;19:441–51.
Purchart L, Kula E. Content of heavy metals in bodies of field ground beetles (Coleoptera, Carabidae) with respect to selected ecological factors. Pol J Ecol 2007;55:305–14.
Ramey CA, Sterner RT. Mortality of gallinaceous birds associated with 2% zinc phosphide baits for control of voles in alfalfa. Int Biodeterior Biodegrad 1995;36:51–64.
Rodriguez-Navarro AB, Gaines K, Romanek C, Masson G. Mineralization of Clapper Rail eggshells from a contaminated salt marsh system. Arch Environ Contam Toxicol 2002;43:449–60.
Roodbergen M, Klok C, van der Hout A. Transfer of heavy metals in the food chain earthworm Black-tailed godwit (*Limosa limosa*): comparison of a polluted and a reference site in The Netherlands. Sci Total Environ 2008;406:407–12.
Roux K, Marra Peter P. The presence and impact of environmental lead in passerine birds along an urban to rural land-use gradient. Arch Environ Contam Toxicol 2007;53:261–8.
Ruuskanen S, Laaksonen T, Morales J, Moreno J, Mateo R, Belskii E, et al. Large-scale geographical variation in eggshell metal and calcium content in a passerine bird (*Ficedula hypoleuca*). Environ Sci Pollut Res 2014;21:3304–17.
Scheifler R, Coeurdassier M, Morilhat C, Bernard N, Faivre B, Flicoteaux P, et al. Lead concentrations in feathers and blood of common blackbirds (*Turdus merula*) and in earthworms inhabiting unpolluted and moderately polluted urban areas. Sci Total Environ 2006;371:197–205.
Schipper AM, Wijnhoven S, Baveco H, van den Brink N. Contaminant exposure in relation to spatio-temporal variation in diet composition: A case study of the little owl (*Athene noctua*). Environ Pollut 2012;163:109–16.
Siebielec G, Smreczak B, Klimkowicz-Pawlas A, Maliszewska-Kordybach B, Terelak H, Koza P, et al. Monitoring chemizmu gleb ornych w Polsce w latach 2010–2012. Puławy. Poland: Instytut Uprawy Nawożenia i Gleboznawstwa Państwowy Instytut Badawczy w Puławach; 2012.
Spahn SA, Sherry TW. Cadmium and lead exposure associated with reduced growth rates, poorer fledging success of little blue heron chicks (*Egretta caerulea*) in South Louisiana Wetlands. Arch Environ Contam Toxicol 1999;37:377–84.
Swaileh KM, Sansur R. Monitoring urban heavy metal pollution using the house sparrow (*Passer domesticus*). J Environ Monit 2006;8:209–13.
Terelak H, Stuczyński T, Motowicka-Terelak T, Maliszewska-Kordybach B, Pietruch C. Monitoring Chemizmu Gleb Ornych Polski w latach 2005–2007. Instytut Uprawy Nawożenia i Gleboznawstwa. Puławy, Poland: National Research Institute in Puławy; 2008 [205 pp.].
Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. Nature 2002;418:671–7.
Van den Steen E, Pinxten R, Jaspers V, Covaci A, Barba E, Carere C. Brominated flame retardants and organochlorines in the European environment using great tit eggs as a biomonitoring tool. Environ Int 2009;35:310–7.
White DH, Finley MT. Uptake and retention of dietary cadmium in mallard ducks. Environ Res 1978;17:53–9.
Zhuang P, Zou H, Shu W. Biotransfer of heavy metals along a soil–plant–insect–chicken food chain: field study. J Environ Sci 2009;21:849–53.