DATA CORRELATION OF MERCURY IN EGGSHELLS AND EGGSHELL MEMBRANES OF WILD BIRDS

Anda Abola[1], Antonija Rimsa[2], Maris Strazds[2], Rita Veilande[1], Gita Revalde[1, 3]
[1]Institute of Atomic Physics and Spectroscopy, Latvia;
[2]Institute of Biology, University of Latvia, Latvia;
[3]Riga Technical University, Latvia
anda.abola@lu.lv, antonija.rimsa@lu.lv, mstrazds@latnet.lv, rita.veilande@lu.lv, gita.revalde@rtu.lv

Abstract. Wild birds are common study subjects as agents of mercury pollution, in particular predominantly fish-eating birds that are at risk for high mercury intake. The Black Stork is one such species in Latvia. Its conservation status is very unfavourable; by the IUCN criteria, it is considered to be critically endangered. Our data encompasses several species of wild birds; however, the bulk of data consists of black stork eggshells. If the target species is endangered, it is highly desirable to reduce the impact caused by the data collection itself. The collection of eggshell remains under nests is one such non-invasive method. Black storks often drop hatched eggshells and the remains of eggs out of their nests. The downside of this method is the fact that one cannot choose a preferable type of artefact to be collected, only what is there. Eggshell remains under nests vary greatly both in size and condition from almost complete eggs to tiny scratches of eggshell. We used an atomic absorption spectrometer with Zeeman correction LUMEX RA-915M paired with its attachment for pyrolytic analysis PYRO-915+ to detect total mercury concentration in our samples. Pyrolytic combustion enables direct measurements without special pre-treatment steps, minimizing sample contamination risk and offering nearly immediate results. To assess our data, we did a statistical analysis using R version 4.2.1. We have previously observed that eggshell membranes contain higher levels of mercury than eggshells themselves. Here we check whether the amount of mercury found in various types of artefacts correlates, using 320 pairs of data for black storks for which both separate measurements were possible. Our data show that the correlation between the two is significant. In addition, we discuss the correlation between eggshells with attached membranes and membranes themselves, as well as between eggshells and eggshells with attached membranes.

Keywords: mercury, black storks, wild birds, eggshells, membranes.

Introduction

Mercury is one of the most toxic elements in the environment. Methylmercury, which is produced during methylation processes in aquatic environments, is one of its most dangerous organic forms. After entering the food chain through tiny organisms, methylmercury starts to bioaccumulate and biomagnify. Because of these processes, even low to moderate mercury concentrations can rapidly reach toxic levels, thus endangering both living organisms and the environment [1].

Because wild birds are top predators in many terrestrial and aquatic environments, and their reproductive systems are highly sensitive to mercury toxicity, they are commonly studied as agents of mercury environmental pollution [2]. A significant role in this process is played by fish, where biomagnification of methylmercury occurs at a high rate [1]. Consequently, these are predominantly fish-eating birds that are at risk for high mercury intake [3].

The Black Stork (*Ciconia nigra*) is one such species in Latvia. Their diet mainly consists of small and average-sized freshwater fish, foraged in streams, fish ponds and similar shallow aquatic environments [4]. The conservation status of the black stork varies across countries in Europe, it is least favourable in the Northern European part of its range (Latvia, Estonia). In Latvia according to the latest assessment it is considered to be critically endangered [5]. The size of the breeding population in Latvia over the last 20 years has decreased more than twice.

Numerous studies have reported results for mercury in the egg parts of various bird species (e.g. [6-10]). Studies of whole eggs have shown that most of the mercury is located in the albumen and yolk and only a small amount is found in shells [9-11]. At the same time, it was concluded, that egg albumen has a strong correlation with shells [6; 9] and membranes [10].

We found only a few previous studies by other authors of mercury concentration measurements in the membranes and eggshells of bird eggs [6; 10]. Brasso et al. in their work [10] indicate, that in terms of mercury assessment, the use of egg albumen is preferable. At the same time, they and Kennamer et al. [9] discuss the advantages of eggshells, such as ease of sample collection, transport and storage, sample availability and less impact on bird populations.

Reducing the impact of data collecting itself is especially desired if the target species is endangered. One such non-invasive technique is gathering of eggshell remnants from beneath nests. Black storks often drop hatched eggshells and the remains of eggs out of their nests [7], meaning they can be collected without climbing to the nests. In addition to reduced interference with the birds themselves, eggshells do not require specific storage conditions. In fact, they can be easily stored for long periods [12], and the use of viable eggs for research purposes is avoided.

The drawback of this approach is that one can only collect what is there, without being able to select a preferred sort of artefact. Eggshell remnants found under nests vary greatly in size and condition, ranging from nearly intact eggs to tiny scratches. Even more – for some eggs, there are only scraps of eggshell available, while for others we can find larger pieces of membrane, but no eggshells. This means that using as many samples as feasible is crucial to increase the spatial coverage of the items gathered and, consequently, to raise the representativeness of data for each given year.

Due to the limited availability of eggshell remains, our interest is to investigate the possible correlations between eggshells and eggshell membranes.

Materials and methods

For this study, we used wild bird eggshells that were collected from 2003 to 2022 in all regions of Latvia. We analysed a total of 397 eggshells, 401 membranes and 209 mixed samples (where the membrane was not separable from the shell). Our data encompass several species of wild birds, namely, the White Stork (*Ciconia ciconia*), the European Roller (*Coracias garrulus*), the Grey Heron (*Ardea cinerea*), the Osprey (*Pandion haliaetus*), the White-tailed Eagle (*Haliaetus albicilla*), the Eagle Owl (*Bubo bubo*), the Lesser Spotted Eagle (*Clanga pomarina*), the Eurasian Woodcock (*Scolopax rusticola*), the Northern Pintail (*Anas acuta*), the Western Capercaillie (*Tetrao urogallus*), the Western Marsh Harrier (*Circus aeruginosus*), the Goshawk (*Accipiter gentilis*), and the Common Buzzard (*Buteo buteo*), however, the bulk of the data consists of black stork eggshells. The use of artefacts of several wild bird species allows us to compare the results between different species.

We labelled all gathered eggshell samples with their respective nest numbers and date of collection and added a description of shell characteristics, including possible hatching status (hatched, unhatched, unknown). We have previously observed that the hatching status may influence how easily membranes can be separated from eggshells [7]. We air-dried eggshells and, if necessary, cleaned them with a soft brush to remove debris.

To prepare samples for measurements, we separated the inner membrane from the eggshell. Sometimes it was impossible, so we measured mixed samples of both eggshell and inner membrane. Immediately before measurements we homogenized our samples using a mortar and pestle.

We did total mercury measurements using an atomic absorption spectrometer with Zeeman background correction LUMEX RA-915M paired with its attachment for pyrolytic analysis PYRO-915+. Pyrolytic combustion enables direct measurements without special pre-treatment steps, minimizing sample contamination risk and offering nearly immediate results. For our sample type, the threshold of detection was approximately 2 $ng \cdot g^{-1}$.

The measurement procedure was carried out as follows: first, we recorded the weight of a sample, then we placed the sample inside the atomizer PYRO-915+, where it was thermally decomposed. Afterwards, we measured the absorption of the mercury 254 nm resonance radiation and calculated the mercury concentration in the sample.

The average mass of our samples was 50-100 mg dry weight (d.w.) for eggshells and 20-30 mg d.w. for inner membranes, as they are a lot less dense than eggshells. We measured each sample 5 times on average, and then calculated the standard deviation to better assess the average value. For calibration and periodical testing we used a certified reference material of mussel tissue – ERM-278k (by the European Commission Joint Research Centre).

We conducted statistical data analysis to look at the relationships between mercury concentrations in different sample compositions: eggshell, membrane, and mixed. All statistical data analyses were done using R version 4.2.1 [13].

Results and discussion

The main problem in using eggshells as biomonitoring artefacts mentioned in [6] and [10] is the low mercury concentrations in eggshells as opposed to egg content. This requires the use of analytical instruments with sufficiently low detection limits. All of our samples had mercury concentrations above the detection limit of 2 $ng \cdot g^{-1}$ of our device.

For this work, we pooled together all measured data for the black stork and evaluated the distribution and mean values for three sample groups – eggshells, membranes and mixed samples (Fig.1). The eggshells had the lowest concentrations, with the average value of 16 $ng \cdot g^{-1}$ and 95% of samples within the range of 3 to 52 $ng \cdot g^{-1}$. For mixed samples, it was difficult to evaluate the contribution of each of the parts, but the concentrations were higher than those of eggshells. Membranes contain more mercury, and the mean value for our samples was 202 $ng \cdot g^{-1}$, with most samples being in the range of 43 to 815 $ng \cdot g^{-1}$. There were also several very high Hg values – over 1000 $ng \cdot g^{-1}$.

Possible sources of mercury contamination in Latvia include anthropogenic activities, such as peat mining, forestry, cement manufacture, illegal dumps, garbage incineration, and military artefacts. In addition, beaver ponds are among the potential natural sources of mercury. Because black storks are migratory birds, mercury may have also been “imported” from other countries [7].

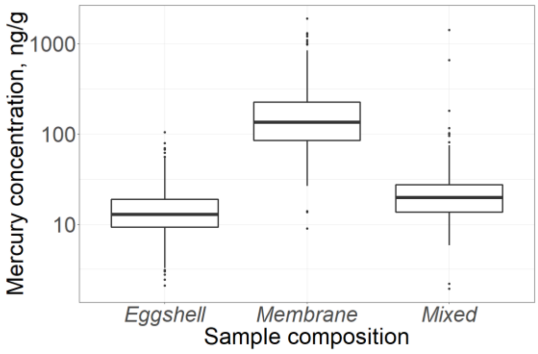

Fig. 1. **Mercury concentration in three sample types of black storks:**
No of samples: eggshells (358), membranes (361), mixed samples (179)

We have previously observed that the total mercury concentration in black stork eggshell membranes is approximately 9 times higher than in shells, using a smaller group of samples [7]. Here, using data from more than 300 sample pairs, we have found that the ratio of mercury in membranes and eggshells is closer to 11:1 respectively, with variations between 4:1 and 30:1. The average value is close to the one for American avocets reported in [6] (13.2:1).

We separated our data on mercury in membranes and in eggshells by the species of wild birds. The largest group, as previously mentioned was for black storks – 361 and 358 samples, respectively. For all the rest of the species, we had from one to ten samples in each group. Preliminary results show that mercury concentrations in other birds’ eggshells are similar to or lower than for black storks. More data is needed to analyse the mercury concentration in relation to bird species.

For the correlation assessment, we selected 320 sample pairs of black stork artefacts for whom we had results for both membranes and eggshells. All Hg concentration values were natural log-transformed before statistical analyses in order to meet the assumptions of normality and homogenous variance for linear regression analysis where possible.

Since our data are non-parametric, we decided to use Spearman’s correlation analysis instead of Pearson’s to look at the correlation between egg membrane mercury concentration and eggshell mercury concentration for black stork samples. Spearman’s correlation analysis on 320 samples showed a statistically significant positive correlation between membrane mercury concentration and eggshell mercury concentration in a sample (ρ = 0.59; S (320) = 2258875; p-value < $2.2e^{-16}$), as shown in Fig. 2. In this case, the value of ρ is positive and greater than 0.5, indicating a moderately strong positive correlation between the two variables.

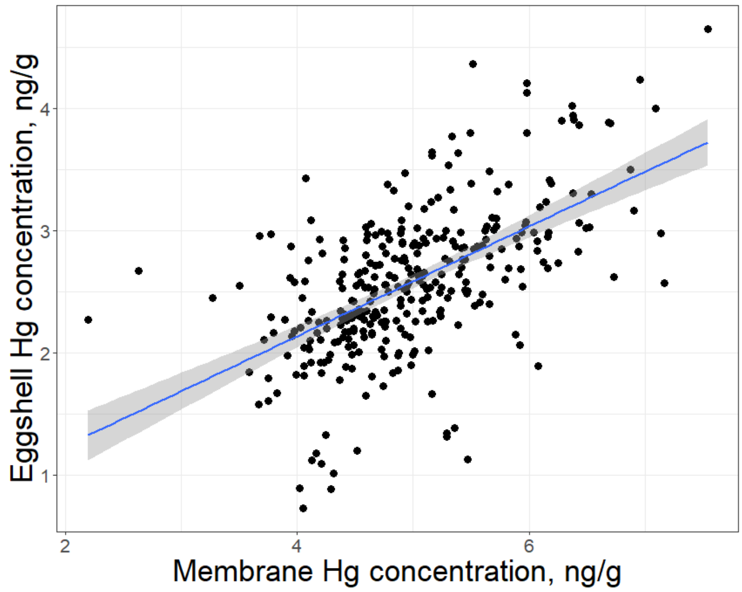

Fig. 2. **Correlation between Hg concentration in membranes and eggshells**

In our collection of eggshells, we had 33 sample pairs obtained from various other wild birds, for whom we had results for both membranes and eggshells. These data points were parametric and linear, so we conducted a Pearson's correlation analysis ($\rho = 0.66$ (95% *CI* 0.40 to 0.82); t (31) = 4.84; p-value < 0.001). The correlation coefficient of 0.66 indicates a moderate positive linear relationship between the two variables, meaning that as the membrane Hg concentration value increases, the eggshell Hg concentration also increases. We have relatively little data on other wild birds that are not black storks at the moment, so sample collection must continue.

We also had 23 black stork samples available, in which the mercury concentrations were measured in all three composition groups: eggshells, membranes, and mixed. We decided to analyse these 23 samples to see whether mercury concentration in mixed composition samples correlates in any way with that of eggshells or membranes.

Firstly, we conducted the Pearson's correlation analysis ($\rho = 0.77$ (95% *CI* 0.52 to 0.89); t (21) = 5.454; p-value < 0.001) to look at the relationship between membrane mercury concentration and mixed mercury concentration, as shown in Fig. 3A.

The results of the correlation analysis indicate that there is a statistically significant strong positive linear relationship between mixed mercury concentration and that of the membrane. The correlation coefficient is 0.77, which suggests that as one variable increases, the other variable also tends to increase.

Additionally, we conducted a linear regression analysis to investigate the relationship between membrane Hg concentration and mixed Hg concentration. The results showed a significant positive relationship between the two variables ($\beta = 0.7278$, $SE = 0.1334$, t (21) = 5.454, $p < 0.01$), indicating that for a one-unit increase in log-transformed mixed Hg concentration, we expect to see a 0.7278 increase in log-transformed membrane Hg concentration, holding all other variables constant. The overall model was significant (F (1,21) = 29.75, $p < 0.01$), and the model accounted for 58.62% of the variance in the log-transformed membrane Hg concentration (adjusted R-squared = 0.5665).

In conclusion, our results suggest that the mixed sample Hg concentration is an important predictor of membrane Hg concentration and that the model provides a good fit for the data. Here is our calculated linear model: log(membrane Hg) = 2.6089 + 0.7278 * log(mixed Hg).

Furthermore, we tested the relationship between eggshell Hg concentration and mixed Hg concentration, using the Pearson's correlation analysis ($\rho = 0.16$ (95% CI -0.27 to 0.54); t (21) = 0.767; p-value = 0.4515), as shown in Fig. 3B. The results suggest that there is not a statistically significant correlation between mixed Hg concentration and eggshell Hg concentration. The weak positive correlation coefficient of 0.1651243 suggests that as one variable increases, the other variable tends to increase slightly, but the confidence interval suggests that we cannot be confident that the true correlation coefficient is significantly different from 0.

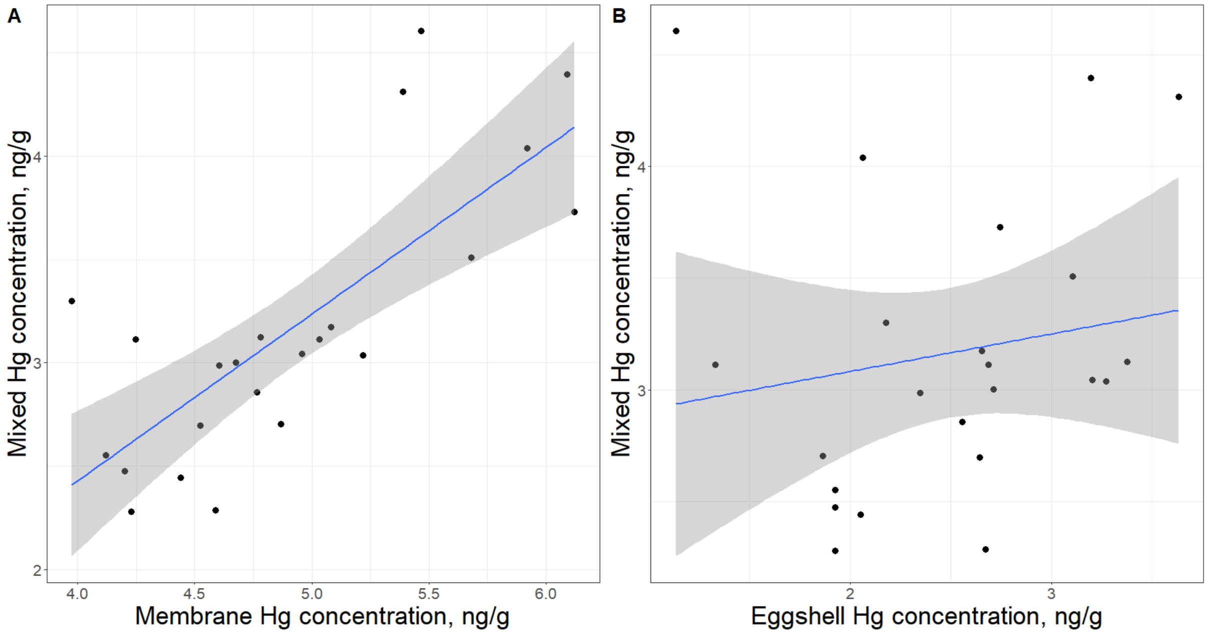

Fig. 3. **Correlation between Hg concentration in membranes and mixed samples (A) and between Hg concentration in eggshells and mixed samples (B)**

Conclusions

Several hundred eggshell samples of various wild birds occurring in Latvia were measured. The average mercury concentration value for eggshells was 16 ng·g^{-1} and for membranes – 202 ng·g^{-1}. The average ratio of mercury in membranes versus shells was 11:1.

The correlation between mercury concentration in eggshells and membranes of black storks has been assessed. Our results show that the correlation is positively moderately strong. In comparing eggshells and membranes to mixed samples, it was found that mixed samples can be used to predict the concentration in membranes. No correlation between mixed samples and eggshells was found.

Preliminary results from other birds separately from the data of black storks were also analysed. The mercury concentrations were similar or lower than those of black storks. The correlation of membranes versus eggshells indicated a positive linear relationship. More data are needed for further analysis.

Acknowledgements

The research was supported by the Latvia Council of Science Project No. lzp-2020/1-0005 and ESF project "Strengthening of the capacity of doctoral studies at the University of Latvia within the framework of the new doctoral model" identification No. 8.2.2.0/20/I/006. We collected all eggshells with respective permits issued by the Nature Conservation Board of Latvia (Dabas Aizsardzības pārvalde). The camera traps used to obtain black stork phenology and other data mentioned in this paper, from 2016 to the present, were provided through the University of Latvia project "Development of the science infrastructure of the national research centre for forest and water resources."

Author contributions

All authors have contributed equally. All authors have read and agreed to the published version of the manuscript.

References

[1] Harding G., Dalziel J., Vass P. Bioaccumulation of methylmercury within the marine food web of the outer Bay of Fundy, Gulf of Maine. PLoS ONE, vol. 13(7), 2018, e0197220.
[2] Ackerman J.T. et al. Avian mercury exposure and toxicological risk across western North America: A synthesis. The Science of the Total Environment, vol. 568, 2016, pp. 749-769.

[3] Evers D. The Effects of Methylmercury on Wildlife: A Comprehensive Review and Approach for Interpretation. The Encyclopedia of the Anthropocene, D. A. DellaSala and M. I. Goldstein, Eds. Oxford: Elsevier, 2018, vol. 5, pp. 181-194.

[4] Janssen, G., Hormann, M., Rohde C. Black Stork (Der Schwarzstorch). First edition. Hohenwarsleben: Westarp Wissenschaften, 2004. 416 p. (In German)

[5] Ķerus V, Dekants A, Auniņš A, Mārdega I. Latvian Breeding Bird Atlases 1980-2017 (Latvijas ligzdojošo putnu atlanti 1980–2017). Rīga: Latvijas Ornitoloģijas biedrība, 2021. 511 p. (In Latvian and English)

[6] Peterson S.H., Ackerman J.T., Eagles-Smith C.A., Hartman C.A., Herzog M.P. A critical evaluation of the utility of eggshells for estimating mercury concentration in avian eggs. Environmental Toxicology and Chemistry, vol. 36(9), 2017, pp. 2417-2427.

[7] Abola A., Strazds M., Veilande R., Gavare Z. Assessing Mercury Pollution Using Black Stork Eggshells. Proceedings of the 13th International Scientific and Practical Conference “Environment. Technologies. Resources.” June 17-18, 2021, Rezekne, Latvia, pp.12-16.

[8] Abola A. et al. Determination of Hg in Biological Samples of Black Storks by Zeeman Atomic Absorption Spectrometry. In Imaging and Applied Optics Congress 2022 (3D, AOA, COSI, ISA, pcAOP), Technical Digest Series (Optica Publishing Group) July 11-15, Vancouver, Canada, 2022, paper JW2A.20.

[9] Kennamer R.A., Stout J.R., Jackson B.P., Colwell S.V., Brisbin I.L. Jr, Burger J. Mercury patterns in wood duck eggs from a contaminated reservoir in South Carolina, USA. Environmental Toxicology & Chemistry, vol.24(7), 2005, pp.1793-800.

[10] Brasso R.L., Abel S., Polito M.J. Pattern of Mercury Allocation into Egg Components is Independent of Dietary Exposure in Gentoo Penguins. Archives of Environmental Contamination and Toxicology, vol. 62, 2012, pp. 494-501.

[11] Aliakbari A., Savabieasfahani M., Ghasempouri S.M. Mercury in egg and eggshell of Whiskered Tern (Chlidonias hybrida) from Anzali wetlands of the Caspian Sea, Iran. Bulletin of Environmental Contamination and Toxicology, vol. 86(2), 2011, pp.175-179.

[12] Xu L.-Q. et al. A 700-year record of mercury in avian eggshells of Guangjin Island, South China Sea. Environmental Pollution, vol. 159(4), 2011, pp. 889-896.

[13] RStudio Team. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, 2020.