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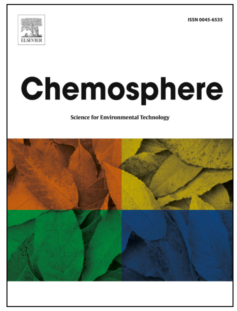

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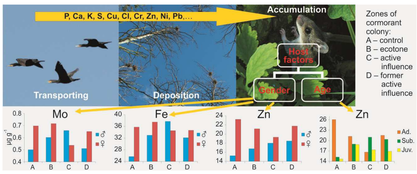

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Accumulation of chemical elements in yellow-necked mice under a colony of great cormorants

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Abstract

This study represents the first investigation into the accumulation of chemical elements in small mammals inhabiting the territory of a great cormorant colony. Trapping was done in the Juodkrantė great cormorant colony, one of the largest colonies in Europe. The accumulation of 20 chemical elements in the bodies (muscle and bones) of yellow-necked mice (*Apodemus flavicollis*) was investigated using the energy-dispersive x-ray fluorescence equipment Spectro Xepos HE. Two groups of positively inter-correlated chemical elements (Mg, Al, P, Ca and Al, S, Cl, K) were identified. The concentrations of five elements differed significantly between mice trapped in different zones of the colony with differing intensities of cormorant influence: the values of K and Cu in *A. flavicollis* increased in line with an increase in the influence of the cormorants, while the concentrations of Rb and Pb decreased. The concentrations of Mn differed between zones, but were not related to the intensity of bird influence. Differences in the concentration of Zn (ANOVA $F = 24.38$; $p < 0.001$), Fe ($F = 4.60$; $p < 0.05$) and Mo ($F = 4.47$; $p < 0.05$) were related to the gender factor, all concentrations being higher in females. The concentrations of Zn were age-dependent, being highest in adult individuals (21.7±4.5 μg g^{-1}) and exceeding those in subadult (19.4±3.4 μg g^{-1}) individuals or juveniles (16.7±1.3 μg g^{-1}). In general, the concentrations of accumulated elements in *A. flavicollis* from the territory of the cormorant colony were lower than in rodents from industrially polluted sites.

Keywords:

Apodemus flavicollis; Phalacrocorax carbo; essential elements; contamination; cormorant colony.

1. Introduction

Atmospheric deposition and anthropogenic activities (agriculture, mining, combustion, industry) release significant quantities of trace elements and heavy metals. Entering water ecosystems, these substances accumulate in hydrobionts or deposit in bottom sediments (Yi et al., 2011; Gajdoš and Janiga, 2015; Hsu et al., 2016). Great cormorants (*Phalacrocorax carbo sinensis*), like other piscivorous birds breeding in colonies, play an important role in transporting nutrients from water to land ecosystems (Osono et al., 2002; Ellis et al., 2006; Gwiazda et al., 2010; Klimaszyk et al., 2015; Otero et al., 2015). Most trees, shrubs and other plants in the territories of cormorant colonies die after few years due to over-fertilization (Garcia et al., 2011) and are replaced by other plant communities (Ayers et al., 2015; Matulevičiūtė et al., 2018). Lichen and fungal communities also change (Osono et al., 2002), as do communities of insects, spiders and lizards (Polis and Hurd, 1996). Most of these changes are related to N and P levels in the soil, which can be increased by 10^4 to 10^5 times (Garcia et al., 2011). Trace elements, including hazardous heavy metals, enter the soil from cormorant excrements (Taraškevičius et al., 2013). Though some trace elements (Al, Fe, Ni, Cu, Zn, Sr, Mo) are harmful at higher concentrations, macroelements (Na, Mg, K, Ca) may be beneficial (Pais and Jones, 1997; Hernout et al., 2016).

Concentrations of heavy metals and trace elements have recently been investigated in different animal taxa, including insects (Aydogan et al., 2017), crustaceans (Gedik et al., 2017), fish (Yi et al., 2011; Benzer, 2017), amphibians (Qureshi et al., 2015), reptiles (Nasri 2017), birds (Kral et al., 2017) and mammals (Lehel et al., 2015; Neila, et al., 2017). Small mammals have been (Wren, 1986) and remain a favourite object for research into metal and trace element accumulation (Martiniaková et al., 2012; Gajdoš and Janiga, 2015; Bortey-Sam et al., 2016;

Khazaee et al., 2016). Accumulation of heavy metals in small mammals has been well documented in polluted areas, including near mines (Phelps and McBee, 2009; Bortey-Sam et al., 2016; Khazaee et al., 2016), power stations (Martiniaková et al., 2010) and paper mills (Gajdoš and Janiga, 2015). Small mammals serve as suitable objects to study the accumulation of heavy metals and trace elements, as the concentrations of the metals in the bodies, organs or tissues of the animals reflect the residues in the soil (Shore and Rattner, 2001; Ieradi et al., 2003; Martiniaková et al., 2011 and references therein). As a research subject, yellow-necked mouse (*Apodemus flavicollis*) was chosen for several reasons. In particular, *A. flavicollis* is the most abundant small mammal species in the territory of the investigated great cormorant colony (Balčiauskas et al., 2016). Additionally, it is characterized by intensive metabolism, a granivorous/insectivorous diet and small individual territories (Butet and Delettre, 2011; Gajdoš and Janiga, 2015). The home range median value for *A. flavicollis* has been identified as 625 m^2 for males and 551 m^2 for females (Vukićević-Radić et al., 2006). *A. flavicollis* is also known as proper biomonitor of metal pollution (Petkovšek et al., 2014), with increased levels of metals in the organism relating to environmental pollution (Martiniaková et al., 2011). In our study, we analysed chemical elements in the muscles and bones of the skinned bodies of *A. flavicollis*. In comparison to internal organs, bones accumulate metals over a longer time period (Martiniaková et al., 2011; 2012) and, as very few *A. flavicollis* individuals live longer than a year, it can be considered to reflect the elemental load of the year of trapping (Martiniaková et al., 2010; Gajdoš and Janiga, 2015).

Various aspects of the influence of great cormorant and other colonial bird colonies on the environment are known already (Ayers et al., 2015; Lafferty et al., 2016). However, investigations into the accumulation of heavy metals and trace elements in small mammals

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inhabiting these colonies are lacking. The investigated colony in Juodkrantė is one of the biggest in Europe, with a maximum number of breeding pairs being 3800 in 2015 (V. Knyva, pers. com). Feeding in both marine and inland waters, including aquaculture, great cormorants are almost purely piscivourous and the estimated biomass of consumed fish in the Juodkrantė colony is ca. 700 tons per year (Pūtys, 2012), with part of this biomass thereafter reaching the ground in the colony in the form of excrement and lost fish, as well as dead chicks during the breeding season.

This study represents the first investigation into heavy metals and trace elements in small mammals occupying the territory of a colony of great cormorants. Research concerning the ecology of mammals in the colonies of great cormorant is still very limited, but decreases in the diversity of the small mammal community and a reduction in abundance have been described in relation to the great cormorant colony in Juodkrantė (Balčiauskienė et al., 2014), along with alterations in the population structure and a decline in body condition (Balčiauskas et al., 2015). Reductions in *A. flavicollis* body weight, body length and index of body condition, as well as changes in skull size and shape, were greatest in the most affected zones of the colony (Balčiauskienė et al., 2015; Balčiauskas et al., 2016). Increased stable isotope signatures in the small mammals, related to the intensity of cormorant influence, show the consequences of biological pollution (Balčiauskas et al., 2016). We hypothesized that such changes may be related to variations in the concentrations of chemical elements in the tissues of the small mammals. To test the hypothesis, we studied the concentrations of 20 elements in the skinned bodies of *A. flavicollis* trapped in different zones with different levels of impact by great cormorants. Differences in accumulation depending on age and gender of mice were evaluated.

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2. Material and methods

2.1 Study site

Small mammals were trapped in a colony of great cormorants situated in the western part of Lithuania near Juodkrantė on the Curonian Spit (WGS 55° 31' 14.22", 21° 6' 37.74"). This colony is the largest in Lithuania and one of the largest in Europe. It is also distinguished by high biological pollution reflected even in small mammals, encompassing all investigated aspects of their biology and ecology (Balčiauskas et al., 2015). The number of breeding pairs reached 3800 in 2015 (V. Knyva, pers. com.) and the area of the colony covers around 12 ha. Four zones with differing levels of colony influence have been defined in the territory (Fig. 1):

Zone A – the control zone. This is outside the colony and there is no direct influence by nesting cormorants on the habitat.

Zone B – the zone of the ecotone. This is located between zones C and D and the surrounding forests that are not influenced by the colony. There are few nests in this zone and the influence of the cormorants is weak.

Zone C – the zone of active influence. This is the active part of the colony with the highest concentration of nests and the strongest influence of the colony.

Zone D – the zone of former active influence. Nests are already abandoned and trees are dead, many of them rotten, fallen and decaying.

2.2 Small mammal sampling

Small mammals were trapped in the middle of September 2015, using snap-trap lines, each consisting of 25 traps spaced 5 m from each other. Traps were baited with brown bread soaked in sunflower oil. Exposition of traps was three days (Balčiauskas et al., 2016). Trapping effort was

750 trap-days. In total, 132 individuals of six small mammal species were trapped: common shrew (*Sorex araneus*), bank vole (*Myodes glareolus*), field vole (*Microtus agrestis*), root vole (*Microtus oeconomus*), harvest mouse (*Micromys minutus*) and yellow-necked mouse (*Apodemus flavicollis*). The dominant species was *A. flavicollis* (70.5% of all trapped small mammals) and the subdominant *M. glareolus* (22.7%). Other small mammal species were insufficiently represented in the various zones of the colony.

Chemical analysis was conducted on the dominant species, *A. flavicollis*. Migration of individuals between zones was investigated in 2013 using live traps and the capture-mark-recapture method. No migration cases were identified.

Before dissection, individuals were weighed (to an accuracy of 0.1 g) and measured with sliding callipers (accuracy of 0.1 mm). The gender and age of the animals were determined during dissection. We used three age categories, adult (ad.), subadult (sub.) and juvenile (juv.), depending on the presence and involution of the *gl. thymus* (involuted in adults, disappearing in subadults, functioning in juveniles) and reproductive status (Balčiauskas et al., 2015). Samples were placed in separate bags, labelled and stored in a freezer at a temperature below -18 °C.

2.3 Study of chemical elements

Chemical elements were analysed in 54 individuals (23 males, 31 females / 21 adults, 21 subadults and 12 juveniles) of the dominant species *A. flavicollis*. We used the skinned body (muscle and bones without intestines, hereafter "body") to register the presence and concentration of the following 20 elements: Na, Mg, Al, Si, P, S, Cl, K, Ca, V, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Mo and Pb. The sampling unit used was body of one individual.

Samples were oven dried at 100 °C for 12 hours, crushed in agate mortars and later pre-mineralized to dry ash at 240 °C to avoid possible ignition and content loss for some volative elements (Markova and Rustschev, 1994; Koh et al., 1999). Ashed samples were milled using the MM 400 mill with zirconium oxide grinding jars and grinding balls (milling time 6 min, frequency 27 Hz). Milled samples were mixed with the Licowax binder (Fluxana) in the proportions of 1.25 g of material and 0.28 g of binder (dilution factor 0.816, as recommended by the equipment manufacturers). Each sample was homogenised and pressed for 3 min using 15 KN (press PP25) to produce 20 mm diameter pellets (Taraškevičius et al., 2017a). The pellets were analysed by energy-dispersive x-ray fluorescence (EDXRF) equipment Xepos HE (Kleve, Germany) using TurboQuant (TQ) II for pellets calibration module as elaborated by the manufacturers. The TQ method combines different procedures: calculation of the mass attenuation coefficient, using the extended Compton model, and final calibration based on fundamental parameters method.

Samples were re-calibrated using standard bovine muscle (BOVM-1) and the International Plant-Analytical Exchange (IPE) program. Four extra sub-samples were taken from each of the IPE Material Samples and from the BOVM-1. Every fifth milled sample (10 extra sub-samples in total) of *A. flavicollis* was divided into two parts to produce an additional second sub-sample of the same primary material. The average values of the variation coefficients of paired sub-samples (RSD) were: < 5% for Na, Mg, Al, P, S, Cl, K, Ca, Mn, Fe, Cu, Br, Rb and Sr; 5–10% for Ni and Zn; 14% for Mo; and 20–23% for Si, V and Pb. The detection limits (μg g^{-1}) of Na, Mg, Al, Si, P, S, Cl, K, Ca, V, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Mo and Pb were 75, 36, 23, 1.5, 2.2, 0.6, 0.8, 1.2, 0.9, 0.3, 0.2, 0.8, 1.0, 0.5, 0.2, 0.06, 0.06, 0.07, 0.2 and 0.2 respectively.

Concentrations of chemical elements were expressed on a wet weight basis in μg g^{-1} (the weight loss on average is 412%).

Preparations were made at the Nature Research Centre (Vilnius) and the analysis of the prepared samples was carried out (Spectro Xepos HE) at the Marine Research Institute, Klaipėda University.

2.4 Statistical analysis

In our analysis, we utilized the mean, range (min–max) and standard deviation of concentrations, Pearson's correlation coefficients and their significance. The normality of the distribution of concentrations was evaluated using Kolmogorov-Smirnov's test (13 out of 20 elements conformed to normal distribution). Based on conformity to normal distribution, parametric tests were used. The influences of multifactors were tested using MANOVA. The influences of zone, gender and age were tested using two-way ANOVA with Wilk's lambda for significance. The Tukey post-hoc test was used to compare multiple independent groups. The minimum significance level was set at $p < 0.05$. We used STATISTICA 6.0 for Windows.

3. Results

3.1 Interaction of host and site factors

The concentrations of chemical elements in *A. flavicollis* depended on which zone of the great cormorant colony they inhabited (MANOVA Wilks $\lambda = 0.04$, $F_{3,53} = 2.90$, $p < 0.001$) and the gender of the animals (Wilks $\lambda = 0.41$, $F_{1,53} = 2.06$, $p < 0.05$), but in most cases did not depend on the age of the animals (Wilks $\lambda = 0.29$, $F_{2,53} = 1.20$, $p = 0.262$). However, animal age did have a significant impact on the concentrations of Zn in *A. flavicollis*.

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Differences in the concentrations of chemical elements did not depend upon interaction of site-based and host factors: age×site (two-way ANOVA $F = 1.14$, $p = 0.225$) and gender×site ($F = 1.16$, $p = 0.264$). However, the interaction of two host factors, i.e., age×gender, did have a statistically significant influence ($F = 1.93$, $p < 0.05$).

3.2 Influence of the great cormorants: the zone factor

Depending on which zone of the great cormorant colony the mice had been trapped in, concentrations of K (ANOVA $F = 6.45$, $p < 0.001$), Mn ($F = 7.04$, $p < 0.001$), Cu ($F = 3.40$, $p < 0.05$), Rb ($F = 14.59$, $p < 0.001$) and Pb ($F = 5.15$, $p < 0.05$) differed in the individuals of *A. flavicollis* (Table S1). The concentration of K was significantly higher in mice from zone D than in zone A (Tukey HSD, $p < 0.01$) and zone C ($p < 0.01$). The concentration of Mn was at its highest in mice from zones A and C. The concentration of Mn in zone A was significantly higher than in zone B (Tukey HSD, $p < 0.01$) and zone D ($p < 0.001$), and the concentration of Mn in zone C was significantly higher than in zone D (Tukey HSD, $p < 0.05$). The concentration of Cu was at its highest in mice from zone C (significantly higher than in zone A, Tukey HSD, $p < 0.05$), though not significantly differing from zone B ($p = 0.09$) and D ($p = 0.71$) (Table S1).

The concentration of Rb in mice from zone A was higher than in zones B, C and D (Tukey HSD, all $p < 0.001$), while the concentration in zone B was higher than in zone D ($p < 0.05$). Similarly, the concentration of Pb was at its highest in mice from zone A (differences from all other zones significant at $p < 0.05$). Thus, the concentrations of K and Cu in *A flavicollis* increased in line with an increased influence of the cormorants, while the concentrations of Rb and Pb decreased (Table S1, Fig. 2).

3.3 Differences related to the gender and age of the mice: the host factor

Differences in the concentrations of Zn (ANOVA $F = 24.38$; $p < 0.001$), Fe ($F = 4.60$; $p < 0.05$) and Mo ($F = 4.47$; $p < 0.05$) were related to the gender factor, all concentrations being higher in females (Table 1). We also found that, for some elements, zone factor had a differing influence on males and females. In females, the concentrations of K (ANOVA, $F = 4.35$, $p < 0.05$) and Cu ($F = 3.12$, $p < 0.05$) differed according to the zone of colony, while this concentration did not differ in males ($F = 1.69$, $p = 0.20$ and $F = 0.73$, $p = 0.16$, respectively). Vice versa, the concentrations of Fe significantly differed according to the zone of the colony in males ($F = 3.57$, $p < 0.05$), but not in females ($F = 0.58$. $p = 0.64$).

Age-related differences were found in the concentrations of Zn ($F = 10.99$, $p < 0.001$). Zn values (21.7 ± 4.5 μg g^{-1}) were highest in the bodies of adult individuals, exceeding those in subadult individuals (Tukey HSD, $p < 0.05$) and juveniles ($p < 0.001$), while values in subadults also exceeded juveniles ($p < 0.05$) (Table 1).

3.4 Inter-elemental correlations

Two groups of chemical elements that positively and significantly correlated between each other within the group were identified in the *A. flavicollis* trapped in the territory of the colony of great cormorants - the first group comprised Mg, Al, P and Ca, while the second group was Al, S, Cl and K (excluding K-Cl, $r = 0.252$, $p = 0.066$) (Fig. 3). Outside these groups, strong significant positive correlations in the concentrations were also found between the pairs Br-Fe ($r = 0.555$, $p < 0.001$) and Sr-Pb ($r = 0.568$, $p < 0.001$). Other correlations between the concentrations of chemical elements are presented in the Table S2.

4. Discussion

Cormorants mediate the transfer of various chemical elements from aquatic to terrestrial ecosystems. The main source of this transfer is bird excreta (Klimaszyk et al., 2015; Otero et al., 2015). Pedogeochemical analysis has shown that guano has a low pH, high levels of P, K and Ca (Breuning-Madsen et al., 2010; Lafferty et al., 2016) and raised concentrations of S, Cl, Cr, Ni, Cu, Zn and Pb (Taraškevičius et al., 2013). As was emphasized by the latter author, “geochemical disbalance can be one of the possible reasons of disturbance in natural ecosystems”. The other possible source of cormorant-borne chemical elements are the fish brought to the colony as food for chicks, some of these being lost and thus reaching the ground (Pūtys, 2012). In fish from water bodies in Lithuania, the accumulation of heavy metals follows the order Cd > Pb > Ni > Zn > Cr > Cu (Idzelis et al., 2008), with concentrations of Cd and Pb frequently exceeding the Maximum Tolerable Limit value of both Lithuania and the European Union (Staniskiene et al., 2006).

In general, the accumulation of trace elements and heavy metals in mammals depends on habitat, available food, season and host factors, such as species, age and gender (Fritsch et al., 2010; Lehel et al., 2015; Neila et al., 2017). Although almost any chemical elements can be detrimental to organisms at high doses, some of these elements (Na, Mg, K, Ca) are not only essential at lower concentrations, but are also frequently lacking in organisms. Amongst these, essential elements such as Al, Fe, Ni, Cu, Zn, Sr and Mo can be toxic in high concentrations, while Pb and Cd may be toxic even at low concentrations (Pais and Jones, 1997; Hernout et al., 2016).

Our study was the first investigation into the accumulation of chemical elements in small mammals inhabiting the territories of great cormorant colonies. The main factor determining the

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concentrations of the chemical elements in *A. flavicollis* was the zone of the colony, this characterizing nest density and bird presence, thus a proxy of bird influence on the local environment. We found a significant increase in the concentrations of K and Cu and a decrease in Rb and Pb in *A. flavicollis* trapped in the zones with increased levels of cormorant impact (Table S1). The overall impact of the influence of cormorants can be considered ambiguous in terms of advantageous or disadvantageous – the increase in the essential K and Cu is positive, as is the decrease in the harmful Pb, but the decrease in the essential Rb is negative. A lack of K can be compensated by heightened Rb intake, extending across all the food web (Nyholm and Tyler, 2000), but a deficiency of Rb is reported as harmful (Gajdoš and Janiga, 2015). In fish at least, however, excess Rb in combination with heightened Pb, Mo and As may act as a spermatogenesis inhibitor (Yamaguchi et al., 2007).

Concentrations of some trace elements and heavy metals in the bodies of *A. flavicollis* were gender dependent: females accumulated significantly higher concentrations of Zn, Fe and Mo. As for the higher concentration of Mo in females, our results confirm those of Gajdoš and Janiga (2015). Higher concentrations of Zn have also been found in female rats (Bortey-Sam et al., 2016), as well as human females (Ziola-Frankowska et al., 2015; Taraškevičius et al., 2017b). Research by Zarrintab and Mirzaei (2017) showed opposite results, there a significantly higher level of Zn was found in male rats. Out of all the analysed elements, only the Zn concentration in *A. flavicollis* was age-dependent in the investigated great cormorant colony. The concentration of Zn increased with age, being highest in adult mice. As well as Zn being involved in the development of sex organs, it is necessary for normal growth and maturation. Additionally, juveniles and pregnant or lactating females have increased requirements for zinc (Roohani, 2013). However, it is known that the Zn concentration in mammals is regulated at constant concentrations and is mostly present within a narrow range (Hernout et al., 2016). From this point of view, our finding of age dependent Zn concentrations in mice from within the cormorant colony requires further attention. Decreased body size of *M glareolus* and common vole (*Microtus arvalis*) have been observed in Pb, Fe, Cu and Zn contaminated areas of Slovakia (Martiniaková et al., 2011), while similar changes in body size and body condition of black-striped mice (*Apodemus agrarius*), wood mice (*A. sylvaticus*) and greater white-toothed shrews (*Crocidura russula*) have been observed in other polluted areas (Sánchez-Chardi et al., 2007a, 2007b; Velickovic, 2007). A decrease in body mass in *A. flavicollis* in the expanding part of the colony typified by fresh nests was also observed in the investigated colony (Balčiauskas et al., 2015). Comparing heavy metal concentrations in *M. glareolus* from the western part of Lithuania (Mažeikytė and Balčiauskas, 2003), the average concentrations of Pb (0.34 μg g^{-1}) and Cu (2.61 μg g^{-1}) in the bodies of these voles were higher than those accumulated in *A. flavicollis* from the great cormorant colony, while the concentration of Ni (0.61 μg g^{-1}) did not differ (Table S1). However, these concentrations are not directly comparable, as *M. glareolus* is known to accumulate Cd, Pb, Cu and Zn in higher concentrations than *A. flavicollis* (Martiniaková et al., 2010, 2011). In addition, we have no data on the possible transfer of heavy metals and other elements with dust, which may have a significant influence on concentrations in small mammals (Metcheva et al., 2001).

Compared to other rodents from industrially polluted sites, the concentrations of the accumulated elements in *A. flavicollis* from the territory of the cormorant colony were lower. However, the results are very inconsistent (Table S3). It is known that the accumulation of heavy metals may differ by up to fivefold between species of shrews, voles and mice in the same territory (Wijnhoven et al., 2007). We do not discuss these differences with respect to the species, site or organs, but it is clear that biological pollution by cormorants is lower than that by industrial outputs. Still however, we found differences in some element accumulations that did depend on the level of the impact of the cormorant colony (Table S1). Concentrations of Pb in *A. flavicollis* from the territory of the great cormorant colony were lower not only than those in rodents from industrially polluted sites, but also in comparison to concentrations of various chemical elements in the tissues of the cormorants (Goutner et al., 2011; Misztal-Szkudlinska et al., 2011). We found two groups of chemical elements in the bodies of *A. flavicollis* with concentrations correlated within the group, namely Mg, Al, P and Ca, plus Al, S, Cl and K (Fig. 3). Interactions between chemical elements can be related to the specific mineral structure of the bone tissue and physiological functions of these elements in the organism (Brodziak-Dopierala et al., 2009). Strong correlations between Mg, P and Ca have been found in human bones (Ziola-Frankowska et al., 2015), while Gajdoš and Janiga (2015) found strong correlations between S and K. Likewise in our case, the correlation between S and K was very strong ($R = 0.808$, $p < 0.001$). Moreover, we complement this group with correlations with Cl and Al.

We may conclude that the accumulation of five out of 20 investigated elements in the bodies of *A. flavicollis* inhabiting the territory of the great cormorant colony depended on the intensity of bird influence. However, as identified by previous research, this is hardly likely to be the sole reason for significant changes in small mammals (Balčiauskienė et al., 2104, 2015; Balčiauskas et al., 2015, 2016). Many ecological factors are changed due to the biological pollution of the cormorant colony, including the food base for the small mammals, the composition of vegetation, the presence of refuges and disturbance by birds. Chemical changes in the colony resulting from the transfer of materials from the aquatic to terrestrial ecosystem work in complex with these other changes.

The main limitation of our work is the sample size. However, the number of small mammals inhabiting the colony is finite and can hardly be bigger. Investigations into other cormorant colonies and other small mammals, such as bank voles (*Myodes glareolus*), will expand the results of this pilot study and may help to gain a deeper understanding of the registered chemical changes.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Table 1. Concentrations of chemical elements (μg g^{-1}) in the bodies of *Apodemus flavicollis* trapped in various zones of the great cormorant colony, according to age and gender groups. Significant differences between groups is presented in bold (ANOVA: * – $p < 0.05$, ** – $p < 0.001$). Superscript letters indicate pairwise age-group differences.

Element	Adults (N=21)		Subadults (N=21)		Juveniles (N=12)		Females (N=31)		Males (N=23)	
	Mean±SD	Min–Max	Mean±SD	Min–Max	Mean±SD	Min–Max	Mean±SD	Min–Max	Mean±SD	Min–Max
Na	708±103	437–929	681±86	523–852	688±69	577–826	706±71	558–852	676±108	437–929
Mg	298±43	199–366	276±43	207–367	298±29	255–360	298±39	227–367	277±41	199–366
Al	81.7±9.3	63.9–96.4	79.8±11.4	59.6–103.9	80.5±10.6	69.4–100.5	80.9±10.3	63.2–103.9	80.4±10.5	59.6–100.5
Si	23.6±21.5	1.5–86.1	29.4±36.0	1.5–149.0	29.3±23.7	2.7–70.1	27.0±23.9	1.5–82.0	27.2±33.5	1.5–149.0
P	4952±528	4106–5996	4772±564	3869–6099	4949±322	4420–5469	4971±496	3973–6099	4760±501	3869–5996
S	2438±328	1738–2977	2280±375	1676–3084	2154±300	1683–2551	2327±335	1710–3084	2294±384	1676–3021
Cl	781±83	628–941	763±74	643–932	761±46	693–831	782±70	643–941	752±73	628–894
K	2721±187	2300–3119	2635±184	2184–2961	2591±206	2242–2880	2670±209	2184–3119	2643±174	2300–2944
Ca	7483±1167	5435–9933	6905±1243	5167–9851	6965±630	5879–7899	7364±1071	5167–9851	6845±1139	5207–9933
V	0.16±0.02	0.12-0.22	0.15±0.02	0.11–0.18	0.15±0.02	0.11–0.18	0.16±0.02	0.11–0.22	0.15±0.02	0.11–0.18
Mn	0.78±0.28	0.48-1.50	0.70±0.34	0.42–2.03	0.88±0.39	0.43–1.75	0.82±0.32	0.43–1.75	0.71±0.34	0.42–2.03
Fe*	34.7±4.2	29.9–42.6	33.4±6.9	22.0–54.9	35.9±3.8	30.3–42.7	**35.8±5.5**	**27.9–54.9**	**32.7±4.8**	**22.0–42.1**
Ni	0.62±0.10	0.38–0.82	0.61±0.12	0.43–0.85	0.6±0.12	0.36–0.78	0.61±0.11	0.36–0.85	0.6±0.1	0.43–0.79
Cu	1.66±0.13	1.37–1.86	1.67±0.12	1.47–1.95	1.74±0.16	1.5–2.03	1.69±0.14	1.46–2.03	1.67±0.13	1.37–2.00
Zn**	**21.7±4.5**^{S,J}	**15.3–23.2**	**19.4±3.4**^{A,J}	**14.5–24.3**	**16.7±1.3**^{A,S}	**14.7–18.6**	**21.3±4.3**	**14.7–28.2**	**17.5±2.3**	**14.5–23.5**
Br	2.38±0.48	1.28–3.17	2.35±0.57	0.88–3.59	2.65±0.37	1.99–3.23	2.41±0.56	0.88–3.59	2.45±0.41	1.58–3.11
Rb	6.27±2.45	3.43–13.49	6.16±2.25	3.76–12.23	6.18±1.15	4.74–8.28	6.35±2.26	3.76–13.49	6±1.93	3.43–12.23
Sr	3.14±1.26	1.91–8.03	2.78±0.51	2.15–4.47	2.60±0.36	2.11–3.28	3.03±1.07	2.08–8.03	2.69±0.49	1.91–3.89
Mo*	0.66±0.19	0.32–1.09	0.61±0.17	0.27–0.88	0.57±0.19	0.24–0.88	**0.66±0.18**	**0.32–1.09**	**0.56±0.16**	**0.24–0.88**
Pb	0.05±0.02	0.02–0.12	0.05±0.02	0.02–0.08	0.04±0.01	0.02–0.05	0.05±0.02	0.02–0.12	0.04±0.01	0.02–0.08

Fig 1. Investigation site (marked by star at inlay map of Lithuania) and location of the zones in the great cormorant colony in Juodkrantė, 2015. Zone A – control, zone B – ecotone between the colony and surrounding forest, zone C – active influence of the colony, zone D – zone of the former influence.

Fig 2. Concentrations of K, Cu, Rb and Pb (μg g^{-1}) in the bodies of *Apodemus flavicollis* from the different zones of the great cormorant colony (age and gender groups pooled). Zone A – control, zone B – ecotone between colony and surrounding forest, C – zone of active influence of the colony, D – zone of former influence.

Fig 3. Intercorrelations between the concentrations of chemical elements in *Apodemus flavicollis* trapped in the territory of the colony of great cormorants (animals from all zones pooled). The correlation between K and Cl ($r = 0.252$, $p = 0.066$) is not shown.

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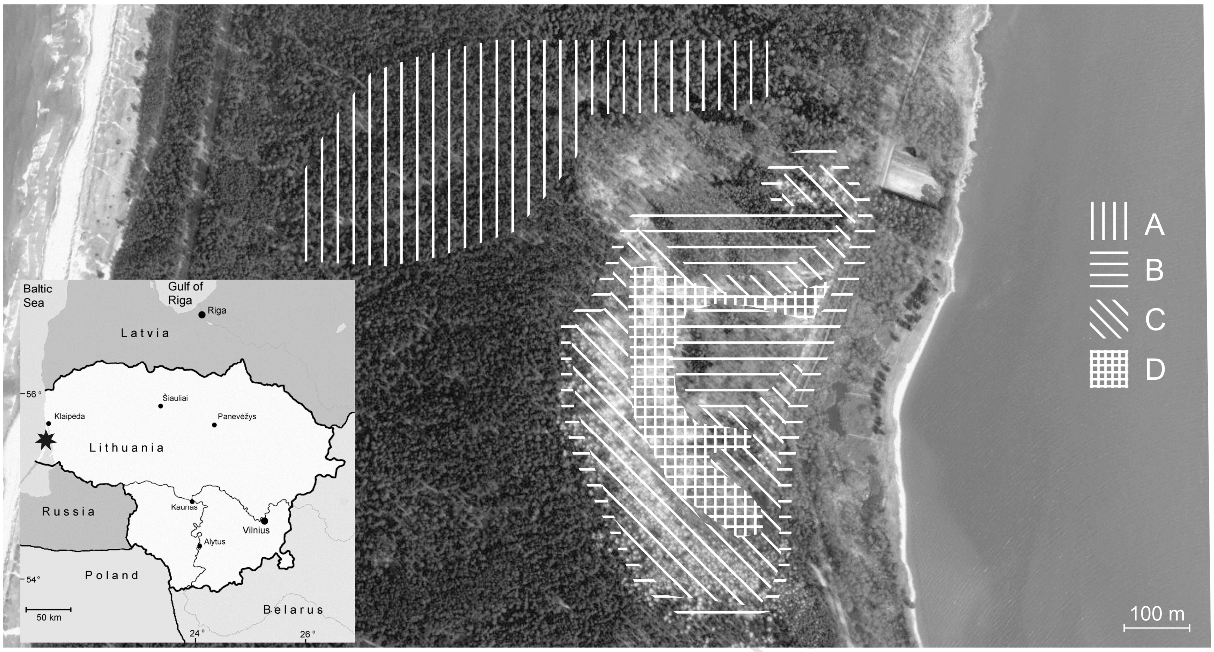
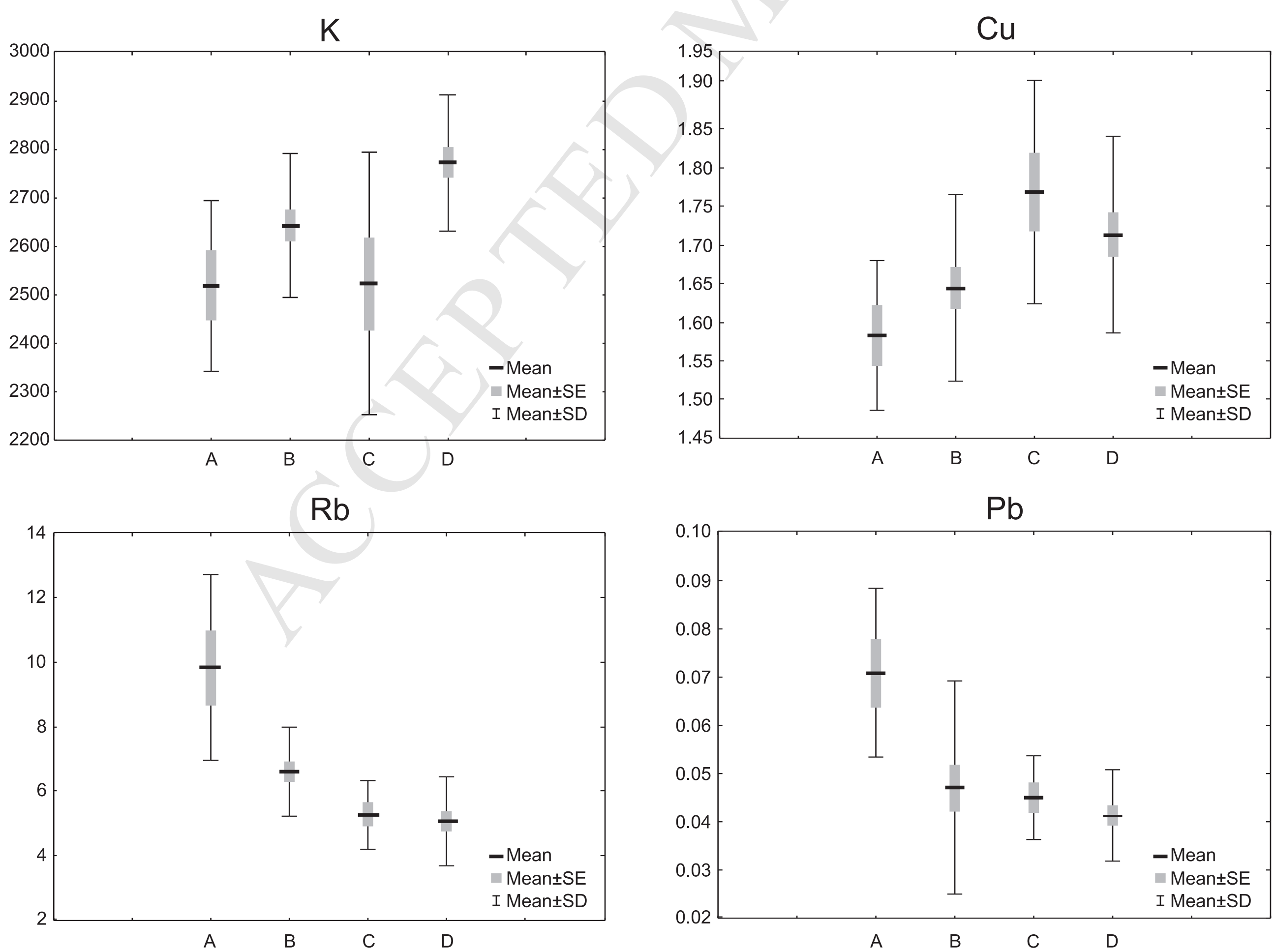


A
B
C
D
100 m
Baltic Sea
Gulf of Riga
Riga
Latvia
56°
Klaipėda
Šiauliai
Panevėžys
Lithuania
Russia
Kaunas
Vilnius
Alytus
Poland
54°
Belarus
50 km
24°
26°



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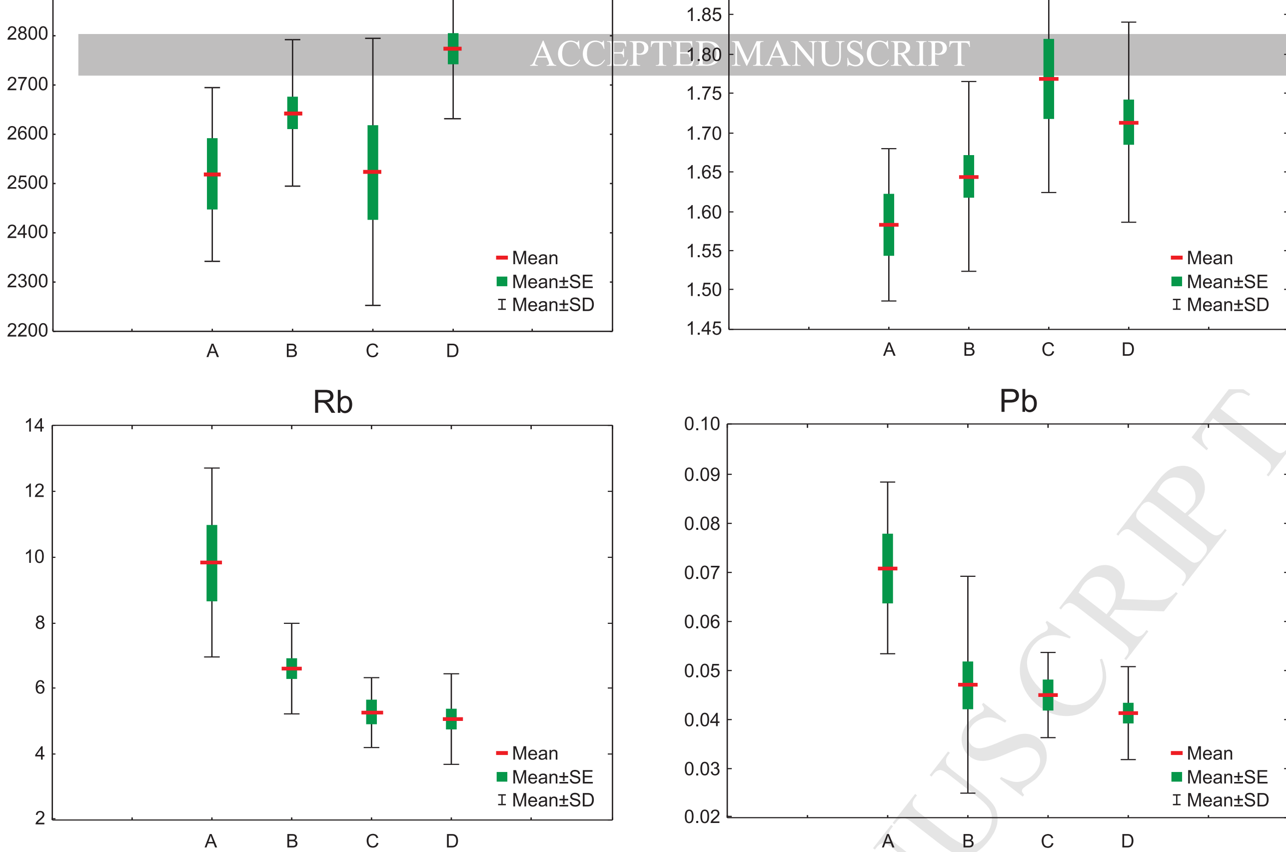

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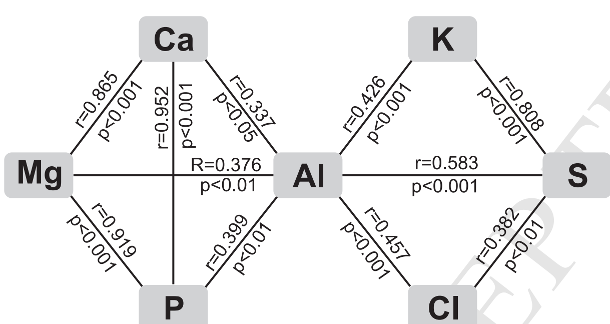

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Highlights

- 20 chemical elements in bodies of *Apodemus flavicollis* from a great cormorant colony were studied.
- Concentrations of K, Mn, Cu, Rb, Pb depended on the intensity of cormorant influence.
- Gender-related differences in concentrations of Zn, Fe and Mo were identified.
- Changes in the chemical environment in the cormorant colony affect small mammals.

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