Urban trace metal contamination is negatively associated with condition and wing morphology in a common waterbird

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ABSTRACT

Urban areas suffer from different forms of environmental pollution by light, noise, and chemicals. Pollution by heavy metals has long been associated with industrialization and urbanization processes, increasing the risk of bioaccumulation and compromising the health, condition, and fitness of urban animals. Here, we aimed to investigate the effects of urbanization on bioaccumulation of six heavy metals (Cu, Ni, Zn, Cd, Pb, and Mn) in the integumentary structures (feathers) of a non-passerine waterbird, the Eurasian coot *Fulica atra*. For this purpose, we quantified and compared heavy metal concentrations in feathers of 300 coots from four pairs of non-urban and urban populations associated with major agglomerations in Poland. We found that concentrations of three heavy metals (Cu, Ni, and Zn) were significantly higher in coots from the urban landscape, compared to individuals from natural or semi-natural non-urban habitats. Elevated heavy metal concentrations were negatively associated with morphology (wing length) and condition (body mass and blood haemoglobin concentration) of coots, and these associations were detected exclusively in the urban landscape. The evidence of elevated heavy metal pollution in the non-urban landscape was limited. Only one heavy metal (Pb) showed a negative association with health parameters of non-urban coots, promoting elevated physiological stress (heterophil/lymphocyte ratios). Our results suggest that heavy metal contamination may be considered an important cost of urbanization processes in wildlife. We argue that mitigation of heavy metal pollution in urban ecosystems should likely increase their sustainability and viability of urban animal populations.

Keywords: condition, heavy metal pollution, morphology, physiological stress, urbanization, waterbirds

1. Introduction

Transformation of natural ecosystems into urban landscape is among the most severe forms of human-induced landscape alterations. Nowadays, global urban development constitutes a major threat to biodiversity, as many organisms are excluded from urban environments (McKinney, 2006; Hahs et al., 2009). At the same time, more and more taxa are effectively colonizing urban landscape and adapting to city life (McDonnell and Hahs, 2015). These adaptations may rely on phenotypic plasticity, which is considered a crucial mechanism in the early phase of urban colonization. Still, they may also have a genetic basis, consistent with microevolutionary processes (Miranda et al., 2013). The adaptations to urban life often co-occur at different axes, including behaviour, physiology, and morphology, responding to diverse ecological and environmental factors (Evans et al., 2009; Lowry et al., 2013; Isaksson, 2020). In fact, the ecological structure of urban ecosystems is unique and usually remarkably different from what organisms experience in their native habitats (Rodewald et al., 2014). Consequently, organisms that disperse into the urban landscape are subject to a whole range of novel selective pressures, including novel biotic interactions (e.g. predation, parasitism, and competition), simplified food webs with abundant anthropogenic food, elevated exposure to acute human disturbance, and different forms of environmental pollution by light, noise, and chemicals (McDonnell and Hahs, 2015).

Pollution by heavy metals has long been associated with industrialization and urbanization processes (Zheng et al., 2023). Heavy metals naturally reside in the soil and their natural release into the environment is limited, only rarely creating the risk of excessive bioaccumulation (Mohammed et al. 2011). In contrast, human activity often produces substantial contamination by heavy metals. The main anthropogenic input of heavy metals into the environment includes atmospheric deposition of industrial and traffic emissions, as well as deposition of technogenic materials linked to housing and industrial activities (Delbecque and Verdoodt 2016). Hence, exposure of wildlife to heavy metals should essentially increase along the urbanization gradient and strong contamination of urban environments may compromise health, condition, and fitness of urban animals (Kekkonen, 2017). However, different heavy metals have different anthropogenic sources, different distribution in the urban space, and different health impact. For example, lead (Pb) is a nonessential metal with no known biological function for living organisms, which causes dysfunction of multiple organ systems, leading to negative health effects that range from chronic and subclinical to acute and fatal (Hydeskov et al., 2024). Historically, Pb was used in many human-made products (e.g. water pipes, paints, gasoline, motor vehicle batteries, and cosmetics), which has increased environmental concentrations of this metal by around 1000 times compared to the reference natural levels (Renberg et al., 2001). Cadmium

(Cd) is another nonessential highly toxic heavy metal, mostly used in alkaline batteries as an electrode component, but also in pigments and coatings (Jaishankar et al., 2014). Cd absorbed from the environment accumulates in the tissues for life and it is known for its adverse effects on the enzymatic systems of cells and oxidative stress (Jaishankar et al., 2014). In recent years, both Pb and Cd have been classified within the top ten positions on the list of chemical substances that pose the most significant potential threat to human health (ATSDR, 2022). Other heavy metals, e.g. copper (Cu), nickel (Ni), zinc (Zn), and manganese (Mn) play important biological functions. However, under elevated exposure they can be hazardous to human and animal health, primarily targeting pulmonary and renal organs, nervous system, and skin, leading to carcinogenesis, neuropathies, and dermatitis (Sharma and Agrawal, 2005).

Although the effects of heavy metal contamination on human health are relatively well understood and acknowledged (Morais et al. 2012), their consequences for wildlife are much less obvious, especially since toxicity levels, clinical effects, and exposure pathways may be taxa- and site-specific (Smith et al. 2007). Thus, to maintain the sustainability of urban ecosystems, it is crucial to evaluate the effects of contamination by different heavy metals on various measures of individual quality across replicated and geographically separated urban and non-urban populations of divergent taxa. However, ecotoxicological studies investigating the effects of urban pollution on birds have often been restricted exclusively to urban populations (thus failing to provide direct comparisons between urban and non-urban landscape), to single geographical locations with no replicated sampling across populations (thus failing to test for the sites-specific effects of urban pollution), or to a narrow spectrum of individual quality measures (thus failing to account for the complex and multi-faceted somatic effects of urban pollution) (e.g. Hofer et al., 2010; Manjula et al., 2015; Hargitai et al., 2016; Joshua et al., 2021). At the same time, taxonomic distribution of such studies has been far from balanced, showing a strong bias towards passerine landbirds (e.g. Markowski et al., 2014; Bauerová et al., 2020; Ross et al., 2024). Consequently, it remains unresolved whether and how general is our understanding of the effects of urban pollution on birds, and whether our knowledge is well applicable to understudied taxonomic groups from different urban microhabitats (e.g. non-passerine waterbirds). To address this question, we used a replicated sampling of feathers across separated urban and non-urban populations of a non-passerine waterbird, the Eurasian coot *Fulica atra* (Rallidae, Gruiformes), quantified concentrations of six heavy metals (Cu, Ni, Zn, Cd, Pb, and Mn), and tested for their associations with multiple phenotypic traits related to individual quality.

Over the last decades, the Eurasian coot has effectively colonized urban areas across Central Europe, being a suitable candidate species to study the effects of urban pollution. As heavy metal contamination and exposure may show local rather than global effects, we replicated our investigation across four pairs of non-urban and urban coot populations associated with major urban agglomerations in Poland. We also investigated associations of heavy metal concentrations with morphology (wing length), condition (size-corrected body mass and total blood haemoglobin concentration), and physiological stress (heterophil/lymphocyte ratio) in breeding coots. We hypothesized that heavy metal concentrations are likely to be higher in urban than non-urban coots due to stronger environmental contamination and exposure. We also hypothesized that elevated heavy metal concentrations in coots may correlate with smaller body size (due to developmental stress), lower condition, and higher physiological stress. Finally, we expected that these negative associations should be primarily apparent in urban rather than non-urban landscape.

2. Methodology

2.1. Study populations and coot capture

Eurasian coots were captured in four pairs of urban and non-urban populations in Poland. Urban populations were located in major urban agglomerations in the country, including Łódź (51°46′37″ N, 19°27′17″E), Poznań (52°24′30″ N, 16°56′01″ E), Katowice (50°15′30″ N, 19°01′39″ E), and Warszawa (52°13′48″ N, 21°00′40″ E) (Fig. 1). Each selected urban agglomeration covered an area of >260km^2 with a human population size ranging between ca. 550 thousand (Poznań) to 2.2 million (Katowice). Sampling sites in these populations were situated in highly urbanized areas, mostly in city centres with compact development and transformed urban green areas with strong human disturbance. The sites (waterbodies) were typically characterized by poor availability of emergent reed vegetation and strong noise/light pollution. The distance between particular agglomerations was 120-260 km. Each urban population was paired with a corresponding non-urban population located at a distance of 15-40 km from the city borders (Fig. 1). Non-urban populations were located in natural and semi-natural landscape, including natural lakes and fishponds. All non-urban sampling sites were characterized by well-developed emergent reed vegetation and little human disturbance, except for hunting activities taking place in the autumn (usually between September and December). These areas were mostly surrounded by agricultural areas, woodlands, and wetlands, with relatively low anthropogenic pressure and low coverage of artificial surfaces (<5% coverage in 2 km buffer zones around each non-urban sampling site; QGIS. 3.32.2, QGIS Development Team 2023, Open Source Geospatial Foundation).

In total, 300 Eurasian coots were captured during the breeding season (March-September) in 2016-2023 across all (four urban and four non-urban) populations. Coots were captured using noose traps made from monofilament fishing line, either directly on the nest during incubation (urban and non-urban sampling sites) or in the breeding territories while feeding on the shore (urban sampling sites). To avoid unintentional recaptures, all captured coots were individually marked with a metal ring and plastic neck collar.

2.2. Morphology, condition, and physiological stress

Upon capture, we collected basic morphological measurements of each individual, including wing length, as a proxy of structural body size. The wing length was measured from the carpal joint (the bend of the wing) to the tip of the longest primary feather using a stopped ruler to the nearest 1 mm. Body mass (measured with an electronic balance to the nearest 1 g) was used as the first proxy of condition. However, since body mass reflects not only condition (quantity of accumulated energy reserves), but also structural body size, we controlled for variation in wing length in the analyses of this trait (see below).

Total blood haemoglobin concentration was used as the second proxy of condition. In general, haemoglobin concentration reflects oxygen-carrying capacity of blood and the aerobic capacity of the organism. Blood haemoglobin concentration has been proposed as the robust indicator of physiological condition in wild birds, as it depends on food availability and diet quality, showing positive correlations with other measures of condition and fitness (reviewed in Minias 2015a). To measure haemoglobin concentration, we punctured the tarsal vein of each captured individual and collected 5 µl of blood into a disposable HemoCue microcuvette (HemoCue, Ängeholm, Sweden). Blood haemoglobin concentration was determined with the azide-methaemoglobin method in a portable photometer HemoCue Hb 201+.

Additional 5 µl of blood was used to prepare smears and quantify leukocyte profiles. Each smear was air-dried, stained using the May–Grünewald–Giemsa method, and scanned at 1000× magnification under a light microscope. A random sample of 100 leukocytes was selected from each smear and classified into five cell types, i.e. heterophils, lymphocytes, eosinophils, basophils, and monocytes. Then, the ratio of heterophils to lymphocytes (H/L ratio) was calculated as a proxy of physiological stress. In birds, stress-induced glucocorticoid release stimulates the so-called white blood cell trafficking, when heterophils transmigrate from the bone marrow into the peripheral blood, while circulating lymphocytes are moved into other body compartments, such as lymph nodes, spleen, or skin (Dhabhar et al. 2012). Since these changes reflect adaptive reorganization of the immune system in a stressful environment, elevated H/L ratios are commonly used as a simple measure of physiological stress in birds and other vertebrates (Davis et al. 2008).

As Eurasian coots are sexually dimorphic in terms of structural body size and body mass (males are on average larger by 6-8% and heavier by ca. 20% than females, Minias 2015b), we aimed to control for between-sex differences in the analyses. Hence, we also collected 50 µl of blood from each captured individual for molecular sexing. Collected blood was stored in 96% ethanol at 5°C. Genomic DNA was extracted using GeneMATRIX Tissue DNA Purification Kit (EURx). Sex-linked chromohelicase-DNA-binding genes were amplified using a protocol developed by Griffiths et al. (1998) and PCR products were separated on a 2% agarose gel. Males and females were identified by one and two bands, respectively, and the sex ratio of our sample was roughly equal (53.2% males).

Because of technical limitations, different traits (i.e. body size, body mass, haemoglobin concentration, and H/L ratios) were measured in a varying number of captured individuals. Final sample sizes ranged from 224 (haemoglobin concentration) to 280 (body mass) individuals (Table 1).

2.3. Heavy metal content in feather samples

In order to measure concentrations of heavy metals, we collected (plucked) feathers from each captured coot, including three body (flank) coverts and one innermost rectrix per individual. All feathers were cleaned from external materials (e.g. dust and plant particles). Still, the measurements of heavy metal content were performed using unwashed feathers, as we aimed to determine the total amount of metals originating both from exogenous and endogenous accumulation (Brait and Filho, 2011; Aloupi et al., 2020). Prior to heavy metal quantification, feather samples (flank feathers and rectrix combined) were oven-dried at 60^{o}C for 24h and then weighed with an accuracy of 0.0001 g. Subsequently, the feathers were digested through thermal mineralization in a mixture of concentrated acids (65% HNO_3 and 70% $HClO_4$) in a proportion of 4:1 v/v (Markowski et al., 2013). After completion of the digestion process, all samples were diluted with deionized water to a total volume of 20 ml and stored in polypropylene metal-free vials at -18^{o}C until further analysis.

Measurements of six selected heavy metals (Cu, Ni, Zn, Cd, Pb, Mn) were conducted using a fast sequential atomic absorption spectrophotometer (Agilent 240 FS AA) with a flame atomizer. Air was used as the oxidizing gas, while acetylene was used as the flammable gas. Heavy metal content was detected at the wavelength of 324.8 (Cu), 232.0 (Ni), 213.9 (Zn), 228.8 (Cd), 217.0 (Pb), and 279.5 (Mn) nm, respectively. We used the lamp current of either 4 (Cu, Ni, and Cd) or 5 (Zn, Pb, and Mn) mA and a slit width of 0.1 (Mn), 0.2 (Ni), 0.5 (Cu and Cd), or 1 (Zn and Pb) nm.

To minimize external metal contamination, all glassware used during laboratory procedures were cleaned in deionized water before each use, soaked in dilute nitric acid for 24 hours, rinsed with deionized water and finally dried (Pandiyan et al., 2020). All reagents used in the protocol were of analytical grade. To ensure quality and accuracy, all sample batches were measured using certified reference material (ERM® - CE278k; mussel tissue) from the Institute for Reference Materials and Measurement (Geel, Belgium) with calibration curves and blind samples included. All metal concentrations in feathers were expressed in µg/g dry weight. Recovery rates for the reference material were within an acceptable margin.

Due to the limited quantity of feather material, we could not always perform the measurements of all six heavy metals in the same individual and the final sample sizes ranged from 234 (Pb and Mn) to 271 (Cu) individuals (Table 1). Sample sizes were well balanced between populations with a mean of 31.8 ± 5.14 (SD) individuals measured for each heavy metal per population. Prior to analysis, we aimed to detect extreme outliers using a conservative criterion of >5 standard deviations (SD) from the mean. In total, we have identified six outlying values in the measurements of Ni, Pb, and Mn concentrations (two outliers per each heavy metal). All identified extreme outliers were removed from the dataset, as being considered technical artefacts. Measurements that retained strong (>1) right skewness after outlier removal (all except Zn) were log_{10}-transformed to improve normality.

2.4. Statistical analyses

All the analyses were run using general linear mixed models (GLMMs), as implemented in the *lme4* package (Bates et al. 2015) developed for the R statistical environment (R Foundation for Statistical Computing, Vienna, Austria). First, we used GLMMs to test for differences in heavy metal concentrations (each used as the response variable in a separate model) between urban and non-urban landscape (landscape urbanization used as a fixed factor). To test if any landscape-level associations were consistent across all sampled population pairs, we also ran models with population entered as fixed factor. Here, pairwise Tukey post-hoc tests were used to test for differences between urban and non-urban landscape within each population pair. Year was included as a random factor in each model to account for inter-annual variation in heavy metal concentrations.

We also used GLMMs to test for associations of body size (wing length), condition (body mass and blood haemoglobin concentration), and physiological stress (H/L ratio) with heavy metal concentrations. All traits representing body size, condition, and physiological stress were used as response variables, while concentrations of heavy metals were entered as covariates in separate models. It was unfeasible to include concentrations of all heavy metals in a single model due to varying sample sizes (Table 1). All models were run separately for urban and non-urban landscape. Year and population identity were entered as random factors, while sex was entered as a fixed factor in each model. Additionally, in the analysis of body mass, haemoglobin concentration, and H/L ratio we included wing length as a covariate to control for variation in structural body size, while in the analysis of haemoglobin concentration and H/L ratio we also included the time (hour) of measurement to control for diurnal variation in these physiological traits.

All GLMMs were fitted using a restricted maximum likelihood (REML) approach and Satterthwaite approximation for degrees of freedom. Effect sizes for the differences in heavy metal concentrations between feather samples from urban and non-urban landscape were calculated as standardized (Cohen's *d*) and unstandardized mean differences (Nakagawa and Cuthill, 2007). Effect sizes for continuous predictors in GLMMs were calculated as semi-partial coefficients of determination R^2 (Nakagawa and Schielzeth, 2013) using the *r2beta* function from the *r2glmm* R package (Jaeger, 2016). All results are reported as means ± SE.

3. Results

3.1. Landscape and population variation

We found significant effects of landscape urbanization on the concentrations of four (out of six) heavy metals in coot feathers. In three of these cases, urban coots showed higher concentrations of heavy metals than coots from non-urban landscape (Cu: $\beta = 0.108 \pm 0.024$, $F_{1, 253.49} = 20.39$, $P < 0.001$; Ni: $\beta = 0.149 \pm 0.043$, $F_{1, 264.86} = 12.10$, $P < 0.001$; Zn: $\beta = 13.86 \pm 1.93$, $F_{1, 248.32} = 7.20$, $P < 0.001$) (Fig. 2; Table S1 in Supplementary Material). The effect sizes of these associations were moderately high, with the average standardized mean difference between urban and non-urban landscape estimated at $d = 0.58$ (max. standardized and unstandardized mean difference for Zn concentration: 0.79 and 13.07 µg/g, respectively). In only one case we found an opposite association with landscape urbanization, as Cd concentrations were higher in feathers of non-urban than urban coots ($\beta = -0.044 \pm 0.017$, $F_{1, 262.27} = 6.99$, $P = 0.009$; Fig. 2; Table S1). However, the effect size of this association was relatively low ($d = 0.29$). Population-level analyses revealed that landscape-level patterns were not consistent across all population pairs, suggesting local effects of heavy metal pollution. For example, Cu concentrations were significantly higher only in Poznań urban area, Ni concentrations only in Warszawa urban area, while Zn concentrations in Poznań and Katowice urban areas, all compared to paired non-urban areas (Tukey post-hoc: all $P < 0.005$; Fig. 2; Table S2 in Supplementary Material). At the same time, there were contrasting patterns of variation in Cd concentrations between different population pairs, i.e. Cd concentrations were higher in Łódź urban area ($P < 0.001$), but lower in Poznań ($P = 0.002$) and Katowice ($P < 0.001$) urban areas, compared to paired non-urban areas (Fig. 2; Table S2). Concentrations of Pb and Mn showed no significant differences between urban and non-urban landscape (Table S1 and Fig. S1 in Supplementary Material).

3.2. Morphology, condition, and physiological stress

In the urban landscape, we found negative associations of wing length with concentration of three heavy metals in feathers, i.e. Cu ($\beta = -8.52 \pm 3.13$, $t = -2.73$, df = 109.93, $P = 0.007$), Ni ($\beta = -6.01 \pm 1.38$, $t = -4.36$, df = 113.45, $P < 0.001$), and Cd ($\beta = -8.76 \pm 4.08$, $t = -2.15$, df = 99.17, $P = 0.034$) (Fig. 3; Table S3 in Supplementary Material). In contrast, no evidence for negative associations of wing length with heavy metal concentrations was found in non-urban landscape (all $P > 0.05$; Table S3). Negative associations of condition traits (body mass and blood haemoglobin concentration) with heavy metal concentrations in coot feathers also prevailed exclusively in urban rather than non-urban landscape. Specifically, we found negative association of body mass (controlled for structural body size) with Zn concentration ($\beta = -1.15 \pm 0.57$, $t = -2.02$, df = 98.75, $P = 0.046$) and negative associations of haemoglobin concentration with Cu ($\beta = -17.14 \pm 8.45$, $t = -2.03$, df = 86.00, $P = 0.046$) and Mn ($\beta = -15.80 \pm 5.95$, $t = -2.65$, df = 71.81, $P = 0.010$) concentrations in feathers of urban coots (Fig. 4; Table S4 and S5 in Supplementary Material). The only significant relationship in non-urban landscape was found for the H/L ratio, which was positively associated with Pb concentration in feathers ($\beta = 0.093 \pm 0.034$, $t = 2.74$, df = 102.00, $P = 0.007$), indicating elevated physiological stress under heavier Pb contamination (Fig. 5; Table S6 in Supplementary Material). No association of physiological stress (H/L ratios) with heavy metal concentrations in feathers was found in the urban landscape (Table S6). Effect sizes (semi-partial R^2) of all significant associations were moderate, indicating that heavy metal concentrations explained 3.7-12.5% of the variance in body size (urban landscape), 3.6-8.2% of the variance in condition (urban landscape), and 6.6% of the variance in physiological stress (non-urban landscape).

4. Discussion

The results of this study provide convincing evidence that urbanization promotes elevated bioaccumulation of heavy metals in the integumentary structures (feathers) of a common waterbird, the Eurasian coot. Specifically, we showed that concentrations of three heavy metals were significantly higher in coots from the urban landscape compared to individuals from natural or semi-natural non-urban habitats. At the same time, we found that elevated heavy metal concentrations were negatively associated with morphology (wing length) and condition (body mass and blood haemoglobin concentration) of coots, but these associations were detected exclusively in the urban (rather than non-urban) landscape. All these results suggest that exposure to elevated heavy metal contamination needs to be considered as an inherent cost of urbanization processes in wildlife. Consequently, we argue that mitigation of heavy metal pollution in urban ecosystems is likely to increase their sustainability and viability of urban animal populations.

Our analyses revealed three heavy metals (Cu, Ni, and Zn), which showed stronger bioaccumulation in urban than non-urban coots. Interestingly, these differences between landscapes had relatively high standardized effect sizes (on average d = 0.58). Consistently, heavy metal concentrations in coot feathers from both types of habitat showed large unstandardized mean differences of up to 13.07 µg/g for Zn concentration (123.37 µg/g vs. 110.30 µg/g for feather samples from urban and non-urban landscape, respectively). The main anthropogenic sources of Cu, Ni, and Zn include industrial activities, mining, and smelting, but also combustion of fossil fuels and phosphate fertilizer production (Cu), food processing (Ni), or waste incineration and traffic (Zn) (Cempel and Nikel, 2006; Rehman et al., 2019; Desaulty and Petelet-Giraud, 2020). Hence, environmental concentrations of these heavy metals are clearly expected to increase substantially in urban agglomerations and industrial areas. Elevated environmental contamination enhances heavy metal bioaccumulation not only in the soft tissues of exposed organisms, but also in their bones, teeth, hair, and other integumentary structures, including bird feathers (Demesko et al., 2019). During the period of growth, feathers are connected with blood vessels and, thus, heavy metals ingested with food can be readily built into their keratin structure (Markowski et al., 2013). Consequently, concentrations of trace elements (such as heavy metals) and other pollutants in bird feathers are expected to reflect the magnitude of bird exposure to these compounds during feather growth (Markowski et al., 2013). After the feather is fully formed, the blood vessels in feathers undergo atrophy and, hence, feathers become physiologically separated from the organism, so the internal accumulation of heavy metals in the feather structure is no longer feasible (Dauwe et al., 2003). Because feathers are moulted seasonally (usually once or twice per year), heavy metals do not accumulate in feather material with age (over lifetime) (Ding et al. 2023), and their internal accumulation in keratin structure indicates relatively recent (in the scale of months) exposure to environmental contamination. After the stage of growth, feathers may also get externally contaminated with heavy metals through direct contact with the environment such as air or water (e.g. Cu, Fe, and Ni) or through preening (Goede and De Bruin, 1986). Although external contamination is likely to be less physiologically risky for the birds than dietary uptake, it reflects even more recent (or current) exposure to heavy metals in the environment. In fact, heavy metals such as Cu, Zn, Pb, and Cd may, at least in some bird species, bioaccumulate more effectively in feathers compared to internal organs, muscles, and bones (e.g. Rodríguez-Álvarez et al., 2022; Ding et al., 2023). For these reasons, feathers have long been recognized as an attractive non-invasive biological material for bioindication purposes (Dmowski and Golimowski, 1993; Burger and Gochfeld, 1995).

Previous analyses of feather material provided consistent evidence for an elevated bioaccumulation of different heavy metals in urban landscape across diverse bird species. For example, elevated Zn concentrations were found in feathers of 11 passerine and non-passerine species from the urban area of Tiruchirappalli, Southern India (Manjula et al., 2015), while elevated Pb concentrations were found in urban common starlings *Sturnus vulgaris* from Georgia, USA (Ross et al., 2024). Another study on great tits *Parus major* and blue tits *Cyanistes caeruleus* in central Poland revealed increased concentrations of Pb and Cd in feathers of nestlings raised in urban parkland compared to birds from suburban forest (Markowski et al., 2014). In our study on the Eurasian coots, Cd was identified as the only heavy metal showing higher concentrations in the feathers of individuals from non-urban than urban landscape. This association was, though, population-specific (different population pairs showed contrasting patterns of variation) and had a low overall effect size. We also recorded no significant differences in the concentrations of Mn and Pb between coots from different landscapes. Taking all this into account, our results reinforce the view that exposure pathways and bioaccumulation rates may not only vary between different metal pollutants, but can also show taxa- and context-specificity. Thus, we conclude that effective and robust biomonitoring of heavy metal pollution should preferably be conducted across a broad spectrum of model and non-model species, always including measurements of diverse metal pollutants.

Our results showed that elevated bioaccumulation of heavy metals is negatively associated with condition indices of urban coots. Specifically, we found that Zn concentrations were negatively associated with size-corrected body mass, while Cu and Mn concentrations showed negative associations with blood haemoglobin concentrations. Heavy metal pollution can have acute effects on multiple organ functions and the overall health of birds. Among the others, intoxication by certain heavy metals may exert direct effects on the cardiovascular system and haematological parameters. Some heavy metals are known to either inhibit haemoglobin production (through inhibition of porphyrin and haem biosynthetic pathway) or increase haemoglobin destruction, causing anaemia in vertebrate animals, including birds (Geens et al., 2010; Ahmed et al., 2022). Hence, reduced haematological values (e.g. total blood haemoglobin concentration) may reflect the direct effects of metal toxicity. For example, Cu may act as a powerful inhibitor of endogenous enzymes, adversely affecting adrenal function and the nervous system, but elevated exposure to Cu may also produce clinical symptoms of anaemia (Anant et al., 2018). This is consistent with our observations of reduced haemoglobin levels in urban coots with high Cu concentrations in feathers. In contrast, Mn toxicity has been primarily associated with dopaminergic dysfunction and changes in neurotransmission, but it also causes cardiovascular dysfunctions, including inhibition of myocardial contraction, blood vessel dilation, and hypotension (O'Neal and Zheng, 2015). Consistently, negative associations of Mn concentrations with various haematological parameters have been reported for different animal taxa (Ahmed et al., 2022), being also consistent with our results.

Detrimental effects of heavy metal intoxication on physiological systems, immunity, and general health are likely to have a negative across-the-board impact on the overall condition (size-corrected body mass), either directly or indirectly, e.g. through decreased food intake (Dauwe et al., 2006). In birds, associations of heavy metal toxicity with condition have been mostly investigated in only few model species, such as the great tit. For example, various haematological parameters (haemoglobin concentration, haematocrit, mean corpuscular volume, and mean corpuscular haemoglobin) were lower in great tits from more polluted industrial (smelting) areas in Belgium (Geens et al., 2010). Heavy metal concentrations in great tit feathers from the direct neighbourhood of the smelter were, though, much (on average 18 times) higher compared to concentrations in coot feathers from our urban study sites. Also, experimental exposure to Pb decreased haematocrit in great tit nestlings (Markowski et al., 2019), while chicks from heavy metal contaminated sites showed reductions in size-corrected body mass (Janssens et al., 2003). However, other studies on this species have failed to find convincing evidence for associations of heavy metal concentrations with basic haematological parameters (e.g. total erythrocyte count) and body mass (Dauwe et al., 2006; Bauerová et al., 2020). At the same time, increasing Pb exposure caused a consistent decrease in blood haemoglobin concentration across four bird species (rock pigeons *Columba livia*, house sparrows *Passer domesticus*, crested pigeons *Ocyphaps lophotes* and white-plumed honeyeaters *Lichenostomus ornatus*) in Australia and the rate of decrease was similar across species (Gillings et al., 2024). Based on these results, blood haemoglobin concentration in birds was recognized as a robust indicator of physiological condition responsive to heavy metal pollution – a conclusion well supported by our study on the Eurasian coot.

We also found that higher concentrations of three heavy metals in coot feathers (Cu, Ni, and Cd) were associated with shorter wing length. The concentrations of heavy metals in feathers are expected to reliably reflect internal (e.g. blood) concentrations of these heavy metals at the moment of feather growth (i.e. during moult), which should, in turn, reflect the level of bird exposure to environmental contamination at this stage (Goede and De Bruin, 1986; Markowski et al., 2013). At the same time, internal concentrations of heavy metals during feather growth may impact developmental homeostasis, i.e. wing morphology and feather length. Experimental research on herring gull *Larus argentatus* nestlings revealed that intraperitoneal injections of lead nitrate solution caused slower growth rates of bill length, tarsus length, and wing bone length (Burger and Gochfeld, 1988). On the other hand, compromised morphological (e.g. feather) development under elevated heavy metal exposure may reflect condition-dependent processes. In birds, the process of feather replacement (moult) is highly demanding in terms of energy and substrates (i.e. proteins) (Murphy, 1996). Under poor condition resulting from metal toxicity, birds may not have the capacity to redirect sufficient resources into keratin synthesis and feather growth, possibly leading to shorter total feather length and wing length. So far, there is convincing evidence for the negative effects of metal toxicity on morphological characteristics in passerine birds. For instance, shorter wing lengths were observed in great tit and blue tit nestlings from areas more polluted with heavy metals (Eeva et al., 2009). Tree sparrows *Passer montanus* from a heavy metal contaminated area in China had smaller body size and higher fluctuating asymmetry in different morphological traits compared to individuals from an unpolluted area (Ding et al., 2022). However, concentrations of different heavy metals in the soft tissues (muscle, kidney, and liver) of adult house sparrows showed contrasting (either positive or negative) associations with different morphological characters (Albayrak and Pekgöz, 2021). Once again, these mixed results advocate for taxa- and context-specific effects of metal toxicity on bird morphology and, thus, we recommend that the scope of ecotoxicological study should extend far beyond well-established model species. Our study on the Eurasian coot, a non-passerine rallid (Rallidae) species, well complements the knowledge gained from research on common passerine birds, such as tits Paridae or sparrows Passeridae. Also, our results clearly showed that the negative associations of heavy metals with both condition and morphology of coots prevailed exclusively in urban habitats and were not observed in natural and semi-natural landscape. Long-term monitoring of marked coots from our study populations indicates that there is virtually no migration between urban and non-urban habitats and natal philopatry of urban individuals is very strong (no evidence of dispersal of urban raised offspring into adjacent non-urban areas; PM pers. observ.). At the same time, coots breeding in urban environment tend to settle in more urbanized landscape during the non-breeding period (Chyb et al., 2021), suggesting that any negative effects of urban-related contamination may be prevalent across their entire annual cycle. Taking all this into account we conclude that elevated exposure to heavy metals should be treated as an important cost associated with colonization of urban areas by wildlife.

We only found one association suggesting that non-urban coots may also be adversely affected by metal toxicity. Specifically, Pb concentrations in coot feathers were positively associated with H/L ratios, which indicate elevated physiological stress in individuals exposed to high Pb levels. Although we did not observe any general (across-population) differences in Pb concentrations between coots from urban and non-urban habitats, it is possible that non-urban individuals may be locally affected by increased Pb pollution. Hunting ammunition and fishing tackle remain among the main anthropogenic sources of Pb pollution in the environment, often leading to the poisoning of waterbirds in their natural habitats (Heig et al., 2014; Newth et al., 2016). In fact, our non-urban sampling sites are known for seasonal (autumn) hunting activity, which may locally increase Pb deposition in water. Positive associations of Pb and other heavy metals with H/L ratios have already been found in some passerine species, including great tits (Bauerová et al., 2020, but see Markowski et al. 2019). In fact, higher H/L ratios in urban birds have often been attributed to increased pollution, indicating that leukocyte profiles can be used as a reliable environmental biomonitoring tool (e.g. Ribeiro et al., 2022).

5. Conclusions

In conclusion, our study showed that urbanization promotes the bioaccumulation of certain heavy metals in waterbird feathers, which may compromise their morphological development and condition. As we found little evidence for negative associations of heavy metal pollution with condition-related traits in Eurasian coots from natural and semi-natural non-urban landscape, we concluded that urban individuals are likely to bear greater costs of heavy metal bioaccumulation. It remains to be investigated, whether and how these costs modulate fitness (reproduction and survival) of urban wildlife and whether urban waterbirds develop any adaptations to compensate for these costs. At the same time, our results suggest that mitigation of heavy metal pollution in urban environment may increase sustainability of urban ecosystems and enhance viability of urban animal populations. We recommend that traditional policies aiming to mitigate heavy metal emissions in the urban environment (e.g. reduction of industrial and traffic emissions) should be effectively combined with innovative green solutions. Most importantly, we suggest that the design or re-design of urban green-blue networks should take into consideration measures that mitigate an influx of heavy metals and other contaminants into urban waters. This may include creation of natural green buffer zones around urban reservoirs and rivers, recreation of nature-oriented water cycle in urban areas, development and implementation of monitoring and protection systems for the natural green-blue spaces in cities and, finally, heavy metal reclamation using nature-based approaches. We suggest that the effective incorporation of these measures into urban planning should help to reduce the adverse effects of heavy metal contamination on urban wildlife (including waterbirds) and enhance urban biodiversity.

Data availability

The data will be made available upon request.

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Table 1

Sample sizes and descriptive statistics for body size, condition, physiological stress, and concentrations of six heavy metals in feathers of Eurasian coots in Poland.

Trait category	Trait	N ind	Mean	SD	Range
Body size	Wing length (mm)	275	213.6	9.1	194-237
Condition	Body mass (g)	280	793.8	121.1	525-1150
	Haemoglobin concentration (g/l)	224	157.5	19.3	101-210
Physiological stress	Heterophil/lymphocyte (H/L) ratio	247	0.90	0.64	0.11-5.53
Heavy metal concentrations (µg/g)	Copper (Cu)	271	7.68	4.77	0.41-29.90
	Nickel (Ni)	269	2.64	3.96	0-25.50
	Zinc (Zn)	251	117.02	16.56	43.73-173.70
	Cadmium (Cd)	269	0.62	0.54	0-2.87
	Lead (Pb)	234	2.21	2.45	0-14.25
	Manganese (Mn)	234	20.16	20.56	0-135.68

Fig. 1. Distribution of non-urban (blue) and urban (orange) sampling sites of Eurasian coots in Poland.

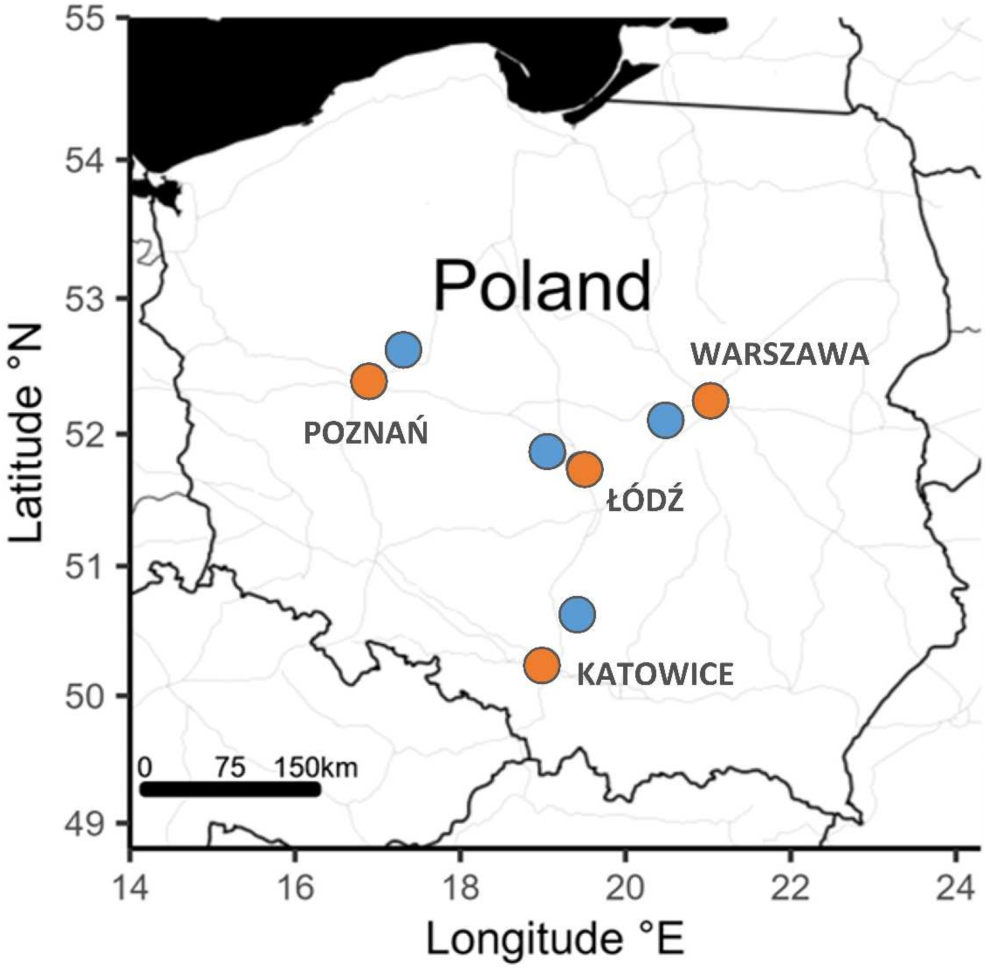


Fig. 2. Concentrations of four heavy metals (Cu, Ni, Zn, and Cd) in feathers of Eurasian coots in Poland showing significant variation between non-urban (blue) and urban (orange) landscape. Landscape- and population-level variation is shown on the left and right panels, respectively. Significant differences are marked with asterisks (* P < 0.05, ** P < 0.01, *** P < 0.001). Central point – mean, box – SE, whiskers – 95% confidence intervals.

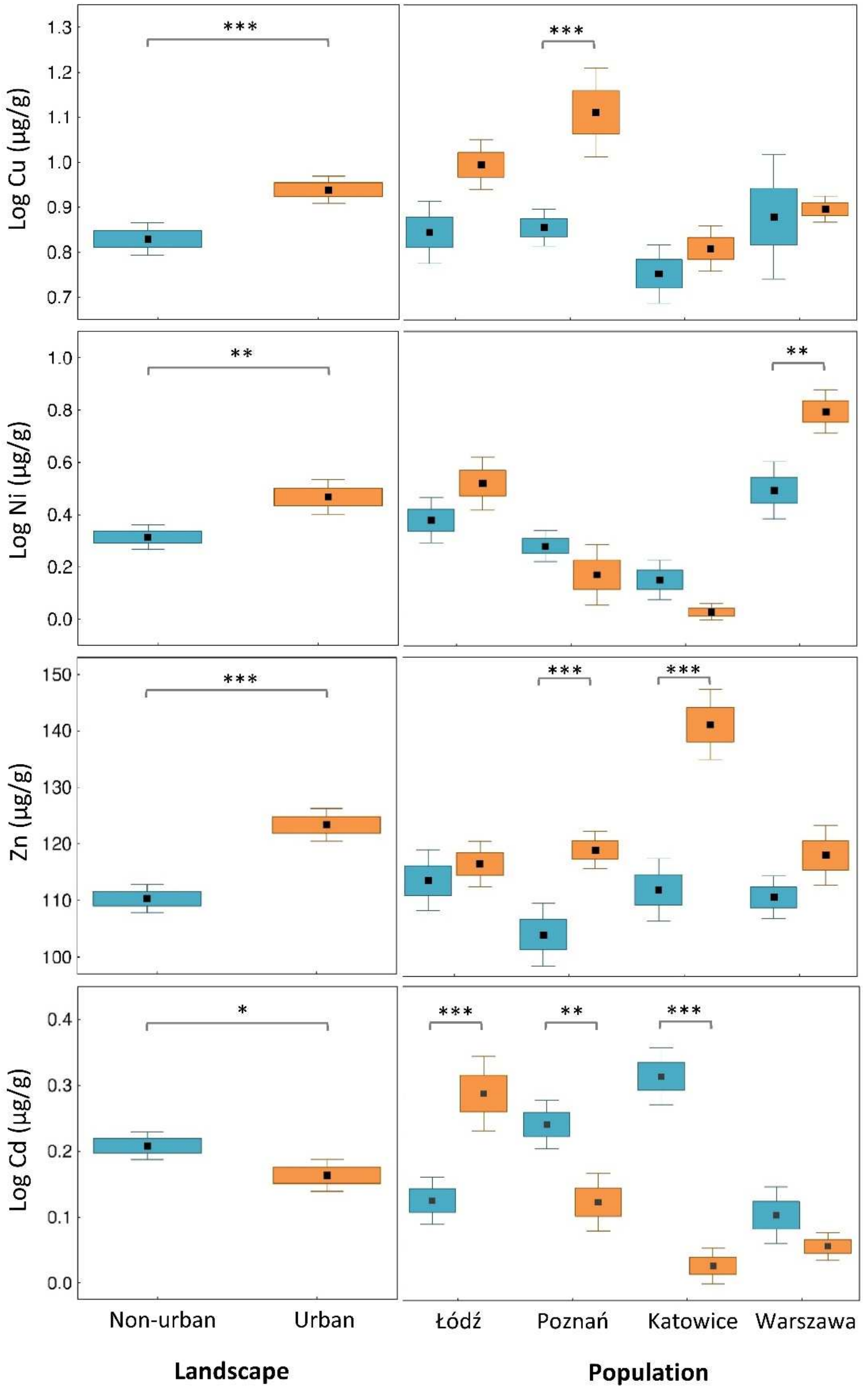
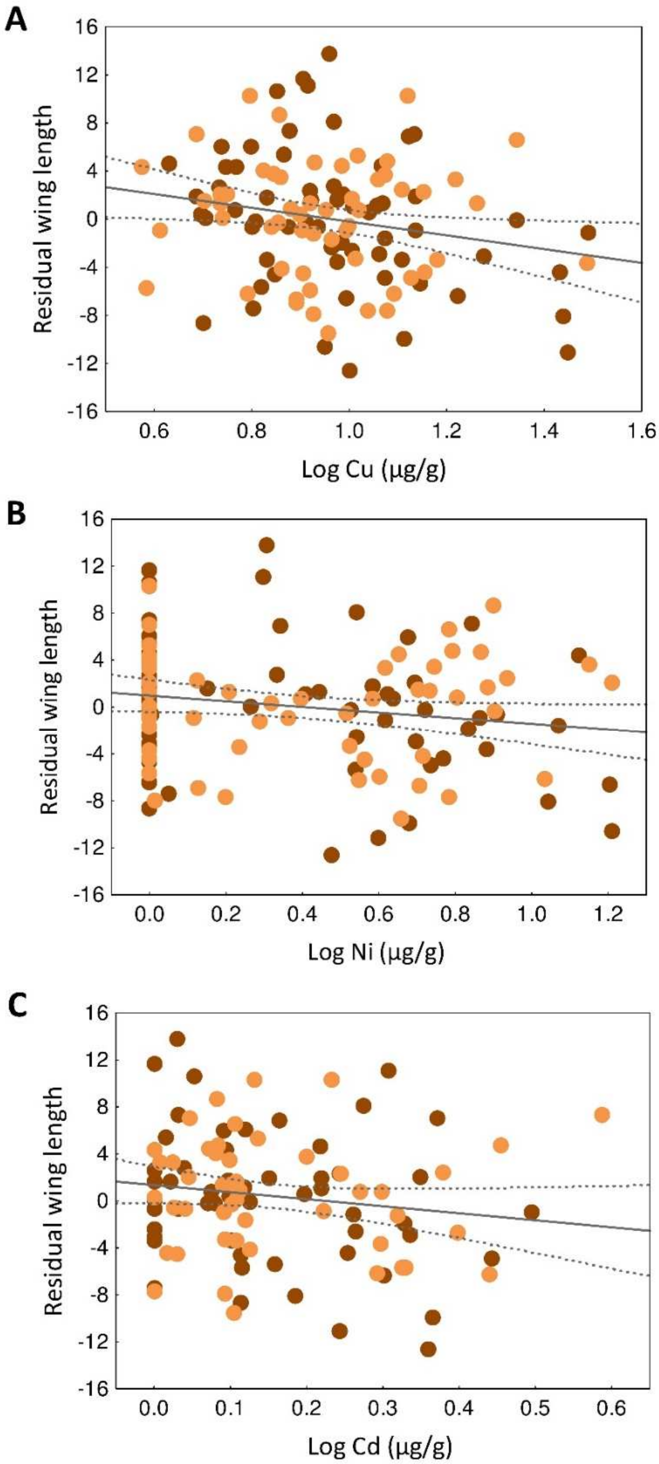
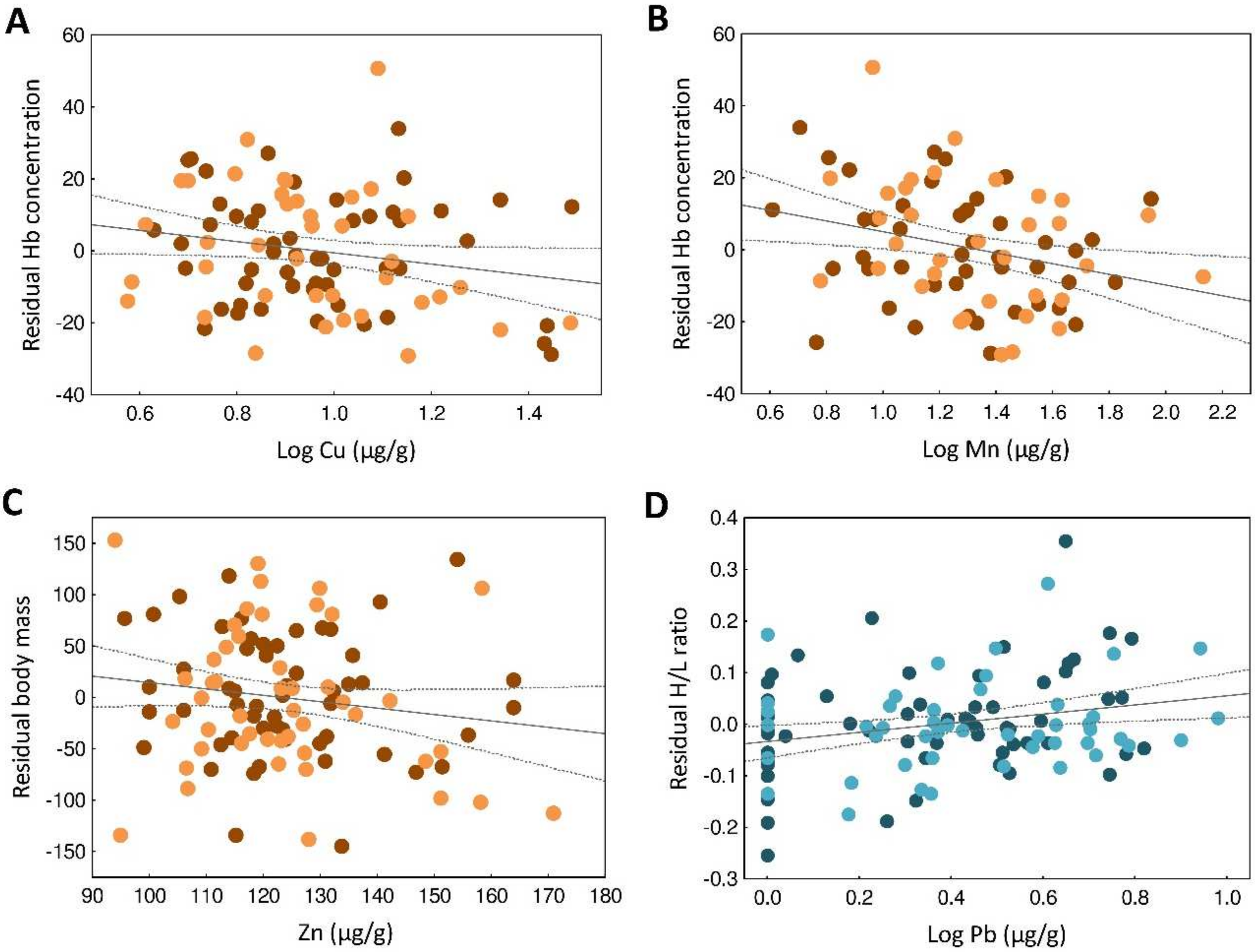


Fig 3. Associations of three heavy metal (Cu, Ni, and Cd) concentrations in feathers of Eurasian coots with wing length, as shown for males (dark orange dots) and females (light orange dots) from the urban landscape. For the purpose of presentation, residuals were calculated to remove the effect of sex from the dependent variable. Regression lines (solid) with 95% confidence intervals (dotted) are presented.



642 **Fig. 4.** Associations of heavy metal concentrations in feathers of Eurasian coots with blood haemoglobin concentration (A: Cu and B: Mn), body mass (C: Zn), and heterophil/lymphocyte (H/L) ratio (D: Pb), as shown for males (dark dots) and females (light dots) from the urban (orange) and non-urban (blue) landscape. For the purpose of presentation, residuals were calculated to remove the effects of sex, wing length, and hour (only Hb and H/L) from the dependent variables. Regression lines (solid) with 95% confidence intervals (dotted) are presented.



648