

## Mercury in Zhongar Alatau (Kazakhstan) and Carpathian mountains (Slovakia): songbirds and mice as indicators

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### Abstract

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Anthropogenic activities have contributed to the increase of heavy metals and to the alteration of their natural cycles in the environment. Mercury (Hg) is now considered to be one of the most toxic elements whose levels need to be monitored in abiogenic and biogenic environmental compartments. It can enter the body of animal in several ways, mainly through contaminated food. In this study, we compared mercury levels in mouse hair and songbird feathers from Zhongar Alatau National Park in Kazakhstan and from national parks in Slovakia. We sampled mice of the genus *Apodemus* and songbirds of three genera – *Parus*, *Phylloscopus* and *Turdus*. Total mercury concentrations were measured using DMA-80. The results showed higher levels of Hg bioaccumulation in Slovakia than in Kazakhstan in both songbirds and mice. The three songbird species from Kazakhstan showed differences due to different feeding niches. High levels were found in thrushes, which are considered insectivorous ground feeders, whereas the lowest mercury concentrations were detected in tits, which are considered more generalist feeders. In Slovakia, the trend was different, with tits having similar levels of Hg to thrushes, a reflection of the different levels of contamination of environmental components. Mice showed overall lower concentrations than birds at both sites.

### Keywords

*Apodemus*, feather, foraging guild, hair, pollution, songbirds

### Introduction

One of the biggest problems facing the world today is environmental pollution. Soil, water and air are contaminated by both natural and anthropogenic sources (UNEP, 2008; HSIAO et al., 2011). In the environment, mercury (Hg) occurs in elemental form (Hg<sup>0</sup>), as monovalent (Hg<sup>+</sup>) and divalent (Hg<sup>2+</sup>) ions, and the organic methylated form of methylmercury (MeHg) is particularly hazardous (GOCHFELD, 2003). Mercury enters living organisms where it

can accumulate. All forms of mercury are highly toxic to humans and the environment (EEA 2011), so monitoring is an important part of understanding these environmental and health threats.

Songbirds and micromammals are suitable bioindicators for monitoring heavy metals. They are abundant, widely distributed, easily captured and responsive to changes in their environment (BELCHEVA et al., 1998; METCHEVA et al., 2003; EDMONDS et al., 2010; HARTMAN et al., 2013, TOWNSEND et al., 2013, BALLOVÁ et al., 2020).

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Most adult mammals shed their fur twice a year (BELTRAN et al., 2018), and the associated phenomenon of seasonal moulting may also involve the detoxification of heavy metals that are displaced into the newly formed hair. Both forms of mercury, ionic and MeHg, bind strongly to the sulfhydryl groups (BJØRKLUND et al., 2017; AJSUVAKOVA et al., 2020) present in the keratin structures of hair (MCLEAN et al., 2009). During the growth of a new coat, the contaminant may be displaced from the bloodstream into the newly developing hair structures (NOËL et al., 2014). The advantage of hair sampling is that it is a non-invasive method (SOBAŇSKA, 2005) and can be collected from both living (STEVENS et al., 1997) and dead individuals, for example from museum exhibits (DAHMARDEH BEHROOZ and POMA, 2021). Similarly, in birds, feathers offer non-lethal alternatives for determining Hg concentrations. However, feather mercury reflects blood and muscle mercury levels at the time of moult (BEARHOP et al., 1999; EVERS et al., 2005), so sampling dates, migration and moulting patterns must be taken into account when analysing Hg in feathers. Both mice and birds provide food for secondary consumers at higher trophic levels where mercury can bioaccumulate and biomagnify, so understanding mercury transfer is important for their conservation.

We examined the level of contamination in the body integuments of mice and three genera of songbirds at the sampling sites. We expect the two sites where the species were collected to have different levels of contamination. The Western Carpathians in Central Europe are known to have high levels of mercury contamination despite their well-preserved ecosystems (MAŇKOVSKÁ et al., 2008; BALLOVÁ et al., 2020). We assume that the area of the second collection site, the Zhongar Alatau National Park in eastern Kazakhstan, Central Asia, is better protected from atmospheric transport of pollutants from distant sources. Since animals, as bioindicators, reflect environmental contamination in their bodies, we expect that animals collected at the second site will have lower levels of contamination. The genera studied also differed in their foraging habits and food preferences. We hypothesise that the level of mercury contamination in feathers and fur will be different due to the different diets. We also investigated the

potential influence of age and morphometrics on mercury contamination in mice.

## Materials and methods

### Site characteristics and sample collection

#### Kazakhstan

In September 2022, bird feathers and mouse hairs were sampled in the Zhongar Alatau National Park in the south-eastern region of Kazakhstan in the Dzungarian Alatau mountain range in Osinovaja N45.40526°, E80.40581° (Fig. 1 left). The climate in the area is characterised as cold and semi-arid, although higher altitudes are colder and wetter due to increased precipitation. The habitat in which the mice and birds were trapped is a forest of wild apple trees (*Malus sieversii*), which is a genetic ancestor of all cultivars of apples.

Sampling of mice was performed using baited live traps of Sherman type. Traps were checked regularly, and in case of successful trapping, species determination, morphometric measurements (front leg, hind leg, ear, tail, body length and body weight) and dorsal hair sample were taken. Animals were released at the site of capture. Collected hair samples were stored in plastic bags before analysis. During the sampling, we captured 28 individuals of wood mouse (*Apodemus sylvaticus*) and one individual of striped field mouse (*Apodemus agrarius*), most of which were juveniles.

Birds were captured in the ornithological mist nets, measured and one tail feather was collected from each individual and stored in plastic bag for mercury analysis. The species captured were *Parus major* (n = 29), *Parus ater* (n = 7), *Parus cyaneus* (n = 6), *Turdus atrogularis* (n = 9) and *Phylloscopus* sp. (n = 7).

#### Slovakia

In Slovakia, comparative species of micromammals and birds were obtained from mountainous Slovak national parks (Fig. 1 right). Micromammals were sampled in October 2022 in the Grapa Nature State Reserve, locat-

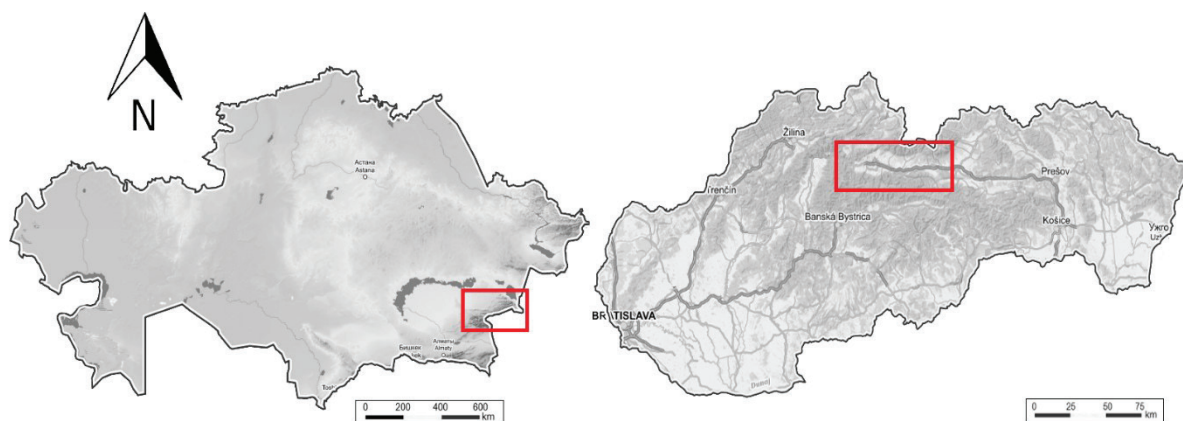


Fig. 1. Map of position of Zhongar Alatau National Park in Kazakhstan (left) and selected national parks in Slovakia (right). Source: Mapy.cz, © Seznam.cz, a.s.

ed in Podspády, Tatra National Park (N 49.286664, E 20.181380) at an altitude of 1,000 m asl. The habitat of the reserve consists of a spruce-fir coniferous forest with the remnants of deciduous beech-fir *Luzulo-fagetum* beech forest and *Tilio-Acerion* forests of slopes, screes and ravines little affected by human activity. Of all the species captured, we sampled 13 individuals of the genus *Apodemus* (*A. sylvaticus* and *A. flavicollis*). This group of mice was supplemented by 14 samples of mice from Tatranská Javorina, Tatra National Park (N 49.266059, E 20.142925), a site 3 km away from Podspády. These mice were trapped during autumn and winter in 2019/2020. Sampling methods were the same as in Kazakhstan.

The bird samples come from mountain areas of Slovak national parks and from mountain villages. As we were not able to capture a sufficiently large representative sample in autumn 2022, we used older samples which were found accidentally dead in the field or as road casualties in the years 1996–2022. All bird samples were deposited in freezer boxes (–18 °C) at the Institute of High Mountain Biology. 46 birds came from Tatra National Park, 14 birds from the Low Tatras National Park and 3 birds from the Malá Fatra National Park. Species were selected to be equivalent to captured species from Kazakhstan, with the same ecological niche and similarities in food preferences. Bird species from Slovakia were *Phylloscopus collybita* (n = 9), *Phylloscopus trochilus* (n = 1), *Parus major* (n = 5), *Parus ater* (n = 3), *Parus caeruleus* (n = 2), *Turdus philomelos* (n = 43).

### Laboratory analysis

Feather and hair samples were analysed using the DMA-80 Direct Mercury Analyzer (Milestone, Italy) to determine the precise concentration of mercury in the samples. The technology is capable of detecting 0.001 ng Hg in the sample. Tissue samples were analysed without any pre-treatment. Samples were weighted on 1 mg accuracy on precision balance (KERN, Germany). Temperatures for the combustion, catalyst and cuvette were set as follows: 650, 615 and 125 °C, respectively. Measurements were performed using the standard method. The certified standard reference material NCS ZC 7001 beef liver (CHNACIS, China) was used to verify the accuracy of the measurement. The recovery was calculated and a single t-test was performed against the reference value. The results of the analysis of the reference material are presented in Table 1. As feather samples contain relatively high amount

of mercury, analyses of blank samples were frequently performed after every fourth sample. This was done to remove any residual amount of mercury and to clean the apparatus. Depending on the detected concentration in blank sample, the next sample was analysed, or another blank run was performed until the detected concentration in the blank sample decreased.

Table 1. Results of the analysis of the certified reference material NCS ZC 7001 beef liver (n = 10)

Certified level (mg kg <sup>-1</sup> )	Detected level (Med ± SD) (mg kg <sup>-1</sup> )	Recovery (%)	Single sample t-test p
0.1800	0.1920 ± 0.0208	106.65	0.1015

### Statistical analysis

Groups of bird genera and mice were compared by location. Homogeneity of variances between compared groups was tested using Levene's test for homogeneity of variances. If the variances were not different, groups were compared by two-sample t-test. In the case of significant result of Levene's test, Welch's F-test of unequal variances was used. Mercury levels in the feathers of three bird genera from the same site were also compared. Because of the limitations of tests comparing multiple groups, the same tests were used to compare two groups.

As the mice captured in Kazakhstan were mostly juveniles, and the mice from the sites in the Slovakia were adults, the age difference may bias the interpretation of the results. To eliminate the effect of age, we compared both groups with data from previous research by ZÁBOJNÍKOVÁ (2022). In this study, adult and juvenile *Apodemus* mice were sampled in a mid-elevation habitat in an agricultural forest in rural/suburban area near Považská Bystrica (PB), northwestern Slovakia. A difference in mercury levels in hair of different age classes was observed. We used these data as a model for the difference between juveniles and adults at the same site. The same tests were used to compare two groups.

A relationship between mercury levels in hair of mice and the body length was tested by correlation. A multivariate technique Principal Component Analysis (PCA) was used to test the influence of different factors on morphometric variables and Hg concentrations. For this analysis

Table 2. Descriptive statistics of Hg concentrations (mg kg<sup>-1</sup>) in bird feathers and mouse hair and their comparison according to locality

	Slovakia			Kazakhstan			Levene's p	t-test p
	Mean ± SD	Min–Max	n	Mean ± SD	Min–Max	n		
<i>Parus</i> sp.	1.6264 ± 1.4472	0.2120–4.6158	10	0.1678 ± 0.1095	0.0346–0.4733	42	<0.0001	<0.0111*
<i>Phylloscopus</i> sp.	0.9439 ± 1.0321	0.0556–2.8577	10	0.3406 ± 0.2780	0.1518–0.8558	6	0.0090	0.1086*
<i>Turdus</i> sp.	1.5363 ± 1.6025	0.2368–8.7558	43	0.8216 ± 0.6017	0.0721–1.8805	9	0.1404	0.1962
<i>Apodemus</i> sp.	0.1198 ± 0.0799	0.0093–0.3968	27	0.0126 ± 0.0161	0.0024–0.0893	29	<0.0001	<0.0001*

\*refers to the use of Welch F test in case of unequal variances.

we used morphometric data of mice from Kazakhstan and levels of Hg in their hair. Factor coordinates and total variance of six factors were calculated.

## Results

Descriptive statistics for mice and all three bird genera studied are summarised in Table 2. Hg concentrations in tits and mice differ significantly between sites (Mann-Whitney test).

When comparing bird genera according to their ecological niche in the same country, Kazakh tits differ significantly from thrushes (Table 3). No significant differences were found among Slovak birds. The comparison of all six groups of birds is shown graphically in Fig. 2.

When comparing hair contamination rates of mice from a single site (PB), a significant difference was observed between adults and juveniles – the fur of adult mice was more contaminated ( $p = 0.1174$ ) (t-test). It was also observed that juveniles from KAZ had similar contamination levels to juveniles from PB. Therefore, if the TJ and

KAZ sites were equally contaminated, adult mice from TJ should have similar contamination rates to adults from PB. However, this was not confirmed – mice from TJ had significantly more contamination in their fur ( $p < 0.0001$ ) (Welch F test) (Table 4, Fig. 3). Therefore, we can say that the contamination rates of the KAZ and TJ sites are different – there is more contamination at the TJ site.

Table 4. Comparison of Hg levels hair of mice of different age from three localities

Couple	Levene's test p	t-test p
Juv KAZ/juv PB	0.8359	0.1174
Juv KAZ/adul TJ	<0.0001	<0.0001*
Juv KAZ/adul PB	0.0005	0.0005*
Adul TJ/juv PB	0.0001	<0.0001*
Adul TJ/adul PB	0.0001	0.0001*
Juv PB/ adul PB	0.0049	<0.0001*

PB – Považská Bystrica (Slovakia); KAZ – Zhongar Alatau (Kazakhstan); TJ – Tatranská Javorina and Podspády (Slovakia). \*refers to the use of Welch F test in case of unequal variances.

Table 3. Comparison of Hg concentrations in couples of birds

Couple	Country	Levene's test p	t-test p
<i>Parus/Phylloscopus</i>	Kazakhstan	0.0007	0.19*
<i>Parus/Turdus</i>	Kazakhstan	<0.0001	0.0115*
<i>Phylloscopus/Turdus</i>	Kazakhstan	0.1745	0.0925
<i>Parus/Phylloscopus</i>	Slovakia	0.4307	0.2416
<i>Parus/Turdus</i>	Slovakia	0.8911	0.8739
<i>Phylloscopus/Turdus</i>	Slovakia	0.6143	0.2714

\*refers to the use of Welch F test in case of unequal variances.

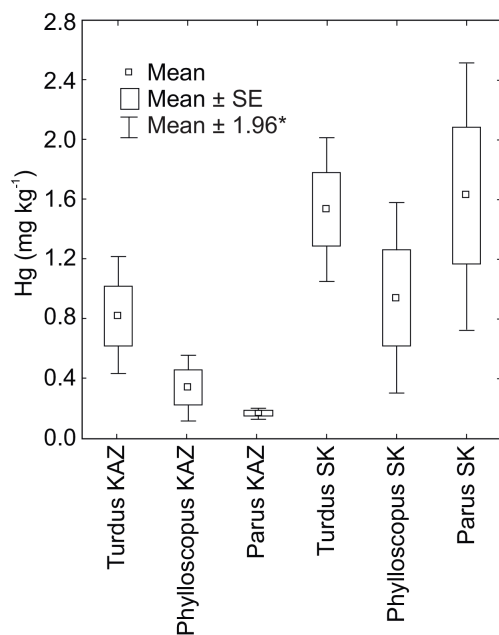


Fig. 2. Hg levels in birds of 3 genera at two catching sites. KAZ – Zhongar Alatau (Kazakhstan); SK – Western Carpathians (Slovakia).

We tested possible synergistic effects of factors influencing body shape and mercury levels in *Apodemus* mice collected in Zhongar Alatau by correlation and PCA. There was no significant correlation between raw data of body length and Hg concentrations ( $p = 0.1720$ ), although the correlation had tendency to be negative ( $r = -0.2656$ ). PCA reveals 6 factors influencing morphometric body shape and mercury levels. Table 5 displays factor score, and percentage of total variance explained by factors.

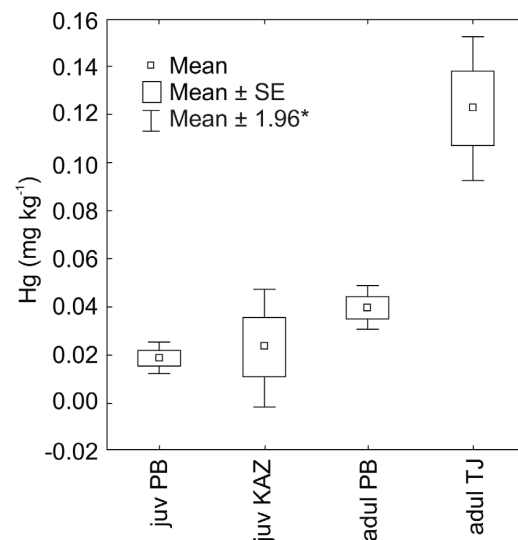


Fig. 3. Comparison of Hg levels in juvenile and adult mice from three sampling localities. PB – Považská Bystrica (Slovakia); KAZ – Zhongar Alatau (Kazakhstan); TJ – Tatranská Javorina and Podspády (Slovakia).

Table 5. Factor coordinates of variables and % of variance explained by the factors influencing morphometric features and Hg concentrations in *Apodemus* mice

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Front leg	-0.7731	-0.3816	0.0633	0.3335	0.3456	0.1484
Hind leg	-0.8322	-0.1309	-0.1964	0.2722	-0.4175	-0.0577
Ear	-0.5415	-0.5051	-0.4786	-0.4687	0.0537	-0.0124
Tail	-0.6882	0.3999	0.4435	-0.2703	-0.0973	0.2954
Body	-0.7226	0.5510	0.1002	-0.0624	0.1628	-0.3658
Hg	0.0058	-0.7047	0.6753	-0.0974	-0.0760	-0.1791
% variance	42.9879	22.9657	15.5725	8.1896	5.6397	4.6445

## Discussion

### Mercury in bird feathers and mouse hair in relation to the environmental pollution at two sites

A comparison of mercury concentrations in both birds and mice showed lower levels of contamination in Kazakhstan's Zhongar Alatau National Park than in the Western Carpathian mountain regions. Although the central and northern regions of Kazakhstan are among the most heavily Hg-contaminated areas due to two former acetaldehyde and chlor-alkali plants in Temirtau and Pavlodar, southern region around Almaty is considered as Hg non-polluted (HSIAO et al., 2011). On the other hand, in the High Tatra Mountains heavy metal pollution, including mercury, has been shown by several studies (e.g. BALLOVÁ et al., 2019, 2020; JANIGA et al., 2016, 2019). Organisms at high altitudes in Slovakia are exposed to higher concentrations of environmental pollutants, most likely due to atmospheric transboundary transfer from the northwestern industrial areas and their subsequent deposition on mountain ridges in the form of relief precipitation during the prevailing northwestern atmospheric circulation. Mercury deposition also varies with forest type and is higher in coniferous forests than in deciduous forests (GRAYDON et al., 2008; RISCH et al., 2012; KOLKA et al., 1999), which actually confirms the higher values in Slovakia, where the forests are mainly coniferous, compared to Kazakhstan's deciduous wild apple tree forests. Furthermore, in Slovakia a large part of the samples come from villages or their surroundings where mercury concentrations may be affected by urban dust, soils, traffic, or combustion (ADACHI and TAINOSHO, 2005; BALL et al., 1998; TRUJILLO-GONZÁLEZ et al., 2016). National parks in Slovakia are much more closely surrounded by industrial and urban areas, where the influence of local and short-range pollution may be more significant, in contrast to the remote and extensive areas of the Kazakh mountains. However, despite higher Hg levels in mice from Slovakia, Hg concentrations in hair samples were well below 20 mg kg<sup>-1</sup>, which is considered a clinically toxic level for terrestrial mammals. The U.S. EPA set a guideline value of 1.0 mg kg<sup>-1</sup> as a reference value for human hair (DIETZ et al., 2011), based on a no observable effect level (NOEL) 12 mg kg<sup>-1</sup>. Even the maximum concentrations found in mice from Tatranská Javorina and Podspády did not exceed this level.

In birds, feather mercury levels of 5 to 40 mg kg<sup>-1</sup> in feathers may be associated with adverse effects (FURTADO et al., 2019). Although the mean Hg concentrations of Slovak and Kazakh birds were below these concentrations, it is important to note how many Slovak individual birds exceeded these values (maximum 8.8 mg kg<sup>-1</sup>), confirming the increased presence of Hg pollution in the Slovak mountains. Birds in montane terrestrial habitats may be at risk (RIMMER et al., 2005), probably as a consequence of higher rates of atmospheric deposition of wet and dry Hg than in lower elevation habitats (VANARSDALE et al., 2005). In Kazakhstan, all samples were spatially restricted, with all bird species occupying the same specific site and habitat, thus providing more reliable results for foraging guild comparisons.

### Mercury in bird feathers in relation to foraging guild

Feathers do not reflect actual Hg levels in the body. Feathers, as inert tissue, are replaced in birds during the summer moulting period. During the formation of new feathers, mercury in the blood is translocated into keratin structures (CONDON and CRISTOL, 2009), therefore, Hg levels in feathers reflects Hg levels in the body at the time of moulting. The oral route is thought to be the main route of Hg entry into the body, so it is necessary to have a thorough knowledge of the dietary habits of the species under study, and thus to take into account dietary guild affiliation when assessing feather Hg levels in different bird species.

### Thrushes – vermivores, frugivores

Thrushes are predominantly ground feeders. They forage on the ground surface in leaf litter and in the upper soil layers (DEL HOYO et al., 2005). The main dietary components of song thrushes are adult and larval insects, lepidopterans, gastropods, myriapods, malacostraca and arachnids (CHAPLYGINA et al., 2019). Earthworms dominate the diet in spring (March–April) (GRUAR et al., 2003) and caterpillars are very abundant in June when they drop from the canopy to the ground to pupate (DAVIES and SNOW, 1965), switching to spiders or snails in the absence of this preferred food. Earthworms are an important species for Hg monitoring because pollutants can enter earthworms through their skin, by ingestion, or both, making them key organisms in the biomagnification processes of soil pollutants (RODRÍGUEZ MARTÍN-DOIMEADIÓS et al., 2015). In July, more than 60% of the diet consists of snails, 30% of

earthworms and the rest of fruit (DAVIES and SNOW, 1965).

The rate of moult in thrushes depends on migration – indigenous species moult more slowly, while migrants have to speed up their moult. The Slovakian thrush samples belonged to the song thrush *T. philomelos*. The moult lasts from mid-July to early September (a period of 50 days), depending on migration, with non-migratory birds moulting more slowly (SNOW, 1969). During the moulting period, earthworms and other invertebrates form the main part of the food web, which is a potential source of contamination. The proportion of fruit in the diet increases, particularly with the onset of autumn, and by September fruit can make up the majority of the total diet (DAVIES and SNOW, 1965).

Among the species analysed from Kazakhstan, the highest Hg concentrations were also found in the feathers of thrushes. In this group of ground-feeding birds, the accumulation of Hg in feathers is explained by the high proportion of animal food, which is thought to accumulate and biomagnify Hg from the soil (TOWNSEND et al., 2013; KNUTSEN and VARIAN-RAMOS, 2020). Birds that forage on the ground and rummage through leaf litter disturb the soil and directly ingest contaminated soil particles.

#### Leaf warblers – insectivores

Both the willow warbler and the common chiffchaff are considered to be leaf gleaners (GREENBERG et al., 1999; BIBI et al., 2019; LAURSEN, 2022), occasionally capable of hunting flying prey in flight (LAURSEN, 2022). During the spring migration, the consumption of beetles on bushes and leaves, ants, flying diptera, wasps and bees was observed (MARCHETTI et al., 1998). Later in the migration, they switch to slow-moving insects, butterfly larvae and herbivorous insects, which are abundant at this time, especially when tree leaves begin to bud (LAURSEN, 1978). During the breeding season, the diet is dominated by leaf-feeding caterpillars (STOSTAD and MENÉNDEZ, 2014), such as Geometridae and Noctuidae (KRIŠTÍN, 1989). Phytophagous invertebrates are more abundant in the diet than zoophagous invertebrates (KRIŠTÍN, 1989). During the autumn migration period (BIBBY and GREEN, 1983), consumption of aphids, spiders, flies, wasps, beetles and hemipterans has been observed in chiffchaff.

As a result of migration, the moulting period of willow warblers is also significantly shortened. The post-nuptial molt of willow warblers begins in June or July and can last 39–45 days (NORMAN, 1990). Depending on the different photoperiods at different latitudes, willow warblers may molt as late as early September (RYZHANOVSKY, 2017). The onset of post-fall molt in adult subspecies of *P. collybita collybita* in Western Europe is in late June and lasts 60–70 days. The amount of mercury found in feathers reflects contamination of food consumed during this period. The pre-nuptial moult of the common chiffchaff lasts from December to January and is incomplete, but rectrices are exchanged (RYZHANOVSKY, 2017). The feather samples from Slovakia were from the spring and summer seasons, i.e. the feathers were produced in winter during the pre-nuptial moult. During this period, chiff-

chaffs were overwintering in a wintering sites. Most chiffchaffs in Europe are short-distance migrants and winter in the Mediterranean (PÉREZ-TRIS et al., 2003). July samples may already be from feathers newly formed during the post-nuptial moult.

Seasonal similarities in diet composition do not predict large differences in feather contamination between seasons. We hypothesise that seasonal differences in contamination are related to differences in contamination of the environment where birds forage during migration, rather than differences in food sources. The Hg content of the feathers of the Zhongar Alatau leaf warblers was in the middle range of the bird groups studied. RIMMER et al. (2010) argue that herbivorous insects, a major food source, typically have higher Hg levels than predatory invertebrates and worms found in the diet of terrestrial foragers.

#### Tits – omnivores, generalists

In terms of foraging methods, tits, especially great tit and blue tit, are leaf feeders, collecting food, mainly invertebrates, on leaves and thin branches in the canopy (ILLERA and ATIENZA, 1995; GIBB, 1954,1960; HARTLEY, 1953). Despite similar food requirements, competition between tit species is minimal, especially during periods of food abundance (GIBB, 1954). During seasonal food shortages in the trees, tits will find alternative sources in shrubs or on the ground. Blue tits are more adapted to foraging by hanging from the branches of shrubs, whereas great tits forage on the ground in an upright position (DÍAZ et al., 1998), most often outside the breeding season when food availability in the canopy decreases (HARTLEY, 1953; GIBB, 1954,1960; BETTS, 1955; SEHHATISABET et al., 2008). Consistent with the moulting period, we hypothesise that Hg concentrations in tit feathers reflect foraging preferences from late spring to autumn. Adult tits undergo a post-breeding moult, the onset of which may overlap with the later breeding season (SOLÍS et al., 2021). As they are non-migratory, feather replacement takes up to a third of the year (115–120 days) (FLEGG and COX, 1969). In late spring and early summer, butterfly larvae are the main component of the tit diet (TÖRÖK, 1985; BETTS, 1955; HARTLEY, 1953), other components are spiders, molluscs, adult beetles, aphids and coccidae (TÖRÖK, 1985; BETTS, 1955; GIBB, 1954), with animal food being predominant during this period (SEHHATISABET et al., 2008). As summer progresses and autumn arrives, the proportion of animal food decreases and the proportion of plant food increases, depending on the availability of seeds and fruits (SEHHATISABET et al., 2008; SORENSSEN, 1981; BETTS, 1955).

While tits in Kazakhstan had significantly the lowest Hg levels, their contamination level in Slovakia was comparable to the highest of the investigated genera – thrushes. The explanation for these differences in Hg levels may be the more diverse habitats from which the tits in Slovakia originate and the associated food resources. These differences may also be due to Hg plume rates; these results also confirm that environmental Hg levels are higher in the Western Carpathians than in the Zhongar Alatau region.

## Mercury in mouse hair in relation to body shape and seasonal diet

The correlation between body length and Hg levels in mouse hair had a negative trend but was not significant. Hg concentrations inversely related to body condition index was also confirmed by KRUK et al. (1997). In contrast, a positive correlation between body weight and Hg concentration in the liver of small rodents was confirmed by DURKALEC et al. (2019). KORSTIAN et al. (2018) looked at the effect of age on Hg concentrations in the fur of bats, with juveniles having less Hg, which they explain by differences in diet. It is possible that higher levels of contamination may lead to reduced overall fitness and reduced motivation and efficiency in foraging. Body size may also affect the overall concentration of contaminants by increasing the dissociation between individual tissues. During fasting, body size decreases, so contaminant concentrations increase in a smaller body, even though the total amount of contaminant remains the same or changes little. This effect has been observed in marine mammals (PETERSON et al., 2018).

However, it should be noted that our data were obtained from mostly juvenile mice. The antagonistic effect of factor 2 on the variables of body length (positive direction) and Hg levels (negative direction) results in smaller juveniles having more Hg and, conversely, larger juveniles having less Hg. The first hypothesis proposed to explain the opposite effects of factor 2, is the dilution of contamination by increasing body size.

The initial dose comes from the mother via placental transfer or lactation (and via eggs in birds) and decreases with growth (DURKALEC et al., 2019). In that case, the younger individuals have more Hg because they have not yet grown enough to dilute it by size. Similar findings have been confirmed for other heavy metals (Pb, Fe, Mg, Mn, Cu, Cr) in *A. sylvaticus* in liver and kidney (SÁNCHEZ-CHARDI et al., 2007), or in fast-growing fish (EAGLES-SMITH et al., 2016). Decreases in Hg concentrations in muscle, kidney, blood and liver of juvenile birds due to massive body and feather growth were confirmed by ACKERMAN et al. (2011). With the onset of adulthood, growth slows down, reducing the efficiency of dilution of contaminants to body size. However, this only applies to metabolically active tissues. Hair, being a more persistent tissue, reflects the concentration at the time of its formation. Therefore, the second hypothesis, that hair concentrations in individuals of different sizes are related to environmental exposures during or just before hair formation, seems more plausible. Immature rodents undergo two or more moults before reaching adulthood, depending on the species (PALOMO et al., 1994). Differences in moult time between juveniles and adults, therefore, reflect differences in environmental contamination at the time of moult, i.e. Hg concentrations were higher in the environment during the moult of juveniles (smaller individuals). Hg contamination in mice also appears to increase with the onset of autumn. Similar conclusions are supported by the study of ZÁBOJNÍKOVÁ (2022), where an increase in Hg concentrations in soft tissues (blood, liver, kidney) of adult

mice was observed with the onset of autumn compared to summer. Again, these changes may be due to a change in dietary preferences. In winter and autumn, mice in a variety of habitats prefer tree seeds as the main component of their diet (ABT and BOCK, 1998; GREEN, 1979; MONTGOMERY and MONTGOMERY, 1990; WATTS, 1968), whereas in spring and summer they supplement their diet with other plant material or animal food. The switch from animal to seed food is probably associated with lower decontamination rates due to reduced protein intake (ADACHI et al., 1992).

## Conclusion

In all three songbird species studied, as well as in mice, skin structures of the Slovak samples contained higher mean Hg. Feather contamination in the three studied genera of Kazakh birds was in the order of thrushes (frugivores/insectivores, preferably ground feeders) > leaf warblers (strictly insectivorous, foliage gleaners) > tits (omnivores, generalists). This ranking assumes higher Hg contamination in the soil. Less Hg is found in tree vegetation and seed producing vegetation is the least affected. These trends in songbird mercury concentrations between foraging guilds are similar to those previously described by TOWNSEND (2013); JACKSON et al. (2015); ACKERMAN et al. (2016, 2018). In Slovakia, however, this order was different. The non-significant differences between the three groups show that the level of contamination in the environmental compartments is approximately the same. The regional difference is most pronounced for tits and their food sources. It is assumed that the food source of tits in the Western Carpathians (larvae, seeds) is contaminated to a higher extent. Their high level of synanthropization may also contribute to the higher mercury levels in their feathers, by direct feeding on contaminated food which can alter their health and body condition (JANIGA, 2022).

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