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The use of principal component analysis to identify factors affecting mercury concentrations in *Apodemus flavicollis* and *Apodemus sylvaticus*

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Abstract. This study deals with the extent to which a spring and autumn moult and the type of seasonally preferred food can affect mercury concentrations in the body of mice. Samples of 5 types of tissues (blood, hair, liver, brain, kidney) were obtained from 102 dead mice of the genus *Apodemus*. Data on the concentration of total Hg in the sample were obtained by a DMA-80 analyzer. PCA revealed 5 factors involved in influencing concentration of Hg in tissues. The origin of these factors and potential explanations for phenomena that are paradoxical are discussed. Comparing the seasonality and effect of the factor that impacts the concentration of Hg in the blood revealed an increase in mercury contamination level in a season where animals experience lower food intake. Exogenous deposition from the environment likely enriches inert hair tissue with mercury following both the spring and autumn moult.

Key words: Apodemus flavicollis, *Apodemus sylvaticus*, mercury, Principal Component Analysis, exogenous deposition, food

Introduction

Mercury (Hg) is not a biogenic element and is highly toxic to organisms in all its forms and compounds. The presence of the contaminant in the environment, particularly in the soil, however, does not necessarily mean that it is available for plant bodies, and the total concentration of the contaminant in the soil is not the same as that available for plants (Rogival *et al.* 2007). Available forms of Hg bind to organic components in the upper horizons of the soil. The overall availability of soil Hg for plants is low, and the concentration of this element in plant organs rarely exceeds the concentration of the surrounding environment. Hg accumulates in the roots, which effectively stabilize its movement, and thus its transport into above-ground parts is prevented (Patra and Sharma 2000). Fungi, unlike plants, can accumulate Hg to a greater extent in the fruiting bodies, especially in the hymenium on the underside of the cap (Kavčič *et al.* 2019). Another crucial factor that participates in determination of the overall concentration of Hg in the plant is foliar uptake directly from the atmosphere (Jiskra *et al.* 2018). The plant absorbs elemental mercury through its leaves along with other gases, and it is photo reduced to another form, which is more persistent in plant organs and is not so easily degradable. During the autumn, Hg deposited in fallen leaves gets into the leaf litter, where it undergoes mineralization and enters the humus layer of soil (Jiskra *et al.* 2018).

In animal bodies, mercury (especially organic form methylmercury) tends to persist in tissues after consumption of contaminated food, caused by biomagnification in the food chain. This phenomenon is well observed in marine (Cardellicchio *et al.* 2002), as well as in terrestrial (Komov *et al.* 2017) ecosystems. Predators at the top of the food chain are exposed to elevated levels of mercury contamination (Mierle *et al.* 2000). Small rodents, which are a source of food for predators, can be an intermediate step transferring contaminants to higher positions in the trophic chain (Gerstenberger *et al.* 2006).

Among tissues, most Hg is accumulated in skin derivatives, such as mammalian hair (Mierle *et al.* 2000, Evans *et al.* 2016) and bird feathers (Dietz *et al.* 2009). Up to 70% of total body burden of Hg can be concentrated in hair (Bearhop *et al.* 2000) and there is up to 250 times more contaminant in hair than in blood (Dietz *et al.* 2009). As animals have a relatively larger surface area given their small dimensions, their fur volume is also proportionally larger compared to body weight. Hart (1956) discusses the effectiveness and limitation of thermoregulatory properties of fur in small and large animals, but little is known about detoxication potential or the possibility of removing contaminants contained in blood. If a mammal is capable of displacing a contaminant from their blood into the newly growing coat, growing of the greater mass of the new coat in small animals may lead to more efficient detoxification than in large animals, whose coat accounts for a smaller proportion of total body weight. Excretion of Hg into feathers and the subsequent decline of concentrations in organs and blood as a result of feather growth has been described in many bird species (e.g. Stewart *et al.* 1994, Caldwell *et al.* 1999, Bearhop *et al.* 2000, Condon and Cristol 2009, Kopec *et al.* 2018, Renedo *et al.* 2018, Janiga and Haas 2019, Albert *et al.* 2021). An effective detoxication mechanism in birds can

displace 70-93% of MeHg from the total body load into feathers (Rimmer *et al.* 2005). The assumption is that this efficiency is due to the large relative weight of feathers in relation to total body weight. However, knowledge of a similar detoxification capability in mammals is limited.

Binding of mercury to keratin in skin derivatives can be an effective adaptation or detoxification mechanism, in which the contaminant is deposited in inert tissues, and thus, more sensitive organs are protected. In birds, Hg is isolated from other tissues and binds to disulphide linkages in feather keratin (Dietz *et al.* 2009). The circulation of Hg in blood and its translocation to tissues is more complex, as birds actively excrete Hg into the feathers during moulting, and then shed those feathers. Once Hg is stored in the feather keratin, further translocation to other parts of the body are no longer possible, as the feather is a metabolically inactive tissue that loses contact with circulation through keratinization (Renedo *et al.* 2018). However, moulting in birds takes place differently from in mammals, as this exchange of feathers does not take place during regular intervals throughout the year, but occurs at different intensities throughout. During periods when feather replacement does not take place, the concentration of Hg in blood rises because excretion is suspended. Thus, during the intermoult period, the only alternative for avian metabolism of Hg is storage in other organ tissues.

Mammals, unlike most birds, have an advantage in that they shed their fur twice per year. New hairs are created next to ones waiting to be shed. Right after the new hair is formed, the hair follicle becomes inactive and is not activated until the next moult (Johnson 1972). The finished hair is a structure of inert cells, thus, it cannot be repaired after damage, and a complete replacement is the only option (Beltran *et al.* 2018). In terms of the annual cycle, the greatest attention is paid to the seasonal changes of summer coat to winter coat (autumn moulting) and winter coat to summer coat (spring moulting).

Material and Methods

Site characteristics and sample collection

Mice samples were collected at the site in the cadastral area of the municipalities of Považská Bystrica, part Považská Teplá, and Plevník-Drienové (N49.15365° E18.47638°, and altitude range of 314-425 m a.s.l.). The vegetation cover in the area consists largely of mixed temperate forests in various succession stages. Extensive small-area timber logging takes place at the locality.

At the site, high concentrations of mercury were not expected. The low-polluted location allows the investigation of the processes caused only by naturally changing seasonal conditions without the influence of anthropogenic pollution.

Concentration data was processed during this examination of seasonal changes in mercury concentrations and the influence of morphometrics. Animals were trapped throughout the year between December 2020 and January 2022. It was not initially intended to analyze internal organs, as the purpose of the study was to collect samples of hair and blood from living animals and release them after sampling. Animals were live-trapped using baited Sherman traps. However, despite the effort to minimalize animal mortality by providing thermal insulation and checking traps as soon as possible, some animals were found deceased in the traps. The carcasses were stored in a freezing box at -20 °C for necropsies. Among all trapped animals, complete data on all five organs (blood, hair, liver, brain, kidney) was obtained from 102 dead mice. These animals are reported herein. No animals were intentionally killed, most of this deceased cohort died overnight in traps or passed unexpectedly during blood collection while narcotized by Isoflurane. Where possible, sex, age (juvenile/subadult/adult), and morphometric data were obtained as well.

Sample preparation and laboratory analysis

Samples were analyzed in a dry state. Blood was dried at room temperature as a drop on Petri dish. Hair was analyzed without any pre-treatment and was not washed. Dissected organs were dried for 24 hours in a laboratory Incubator IF 160 Plus (Memmert, Germany) at 50 °C. The FAN was set to 20%. The weight of each sample was determined by a KERN 770 balance (KERN, Germany). Concentration of Total Hg was detected by 2-cell DMA-80 (Milestone, Italy) with nickel boats. The temperature settings were as followed: temperature for combustion 650 °C, for catalyst 615 °C, cuvette temperature 125 °C. The Certified standard reference material Beef liver NCS ZC 7001 (CHNACIS, China) was used to ensure the accuracy of the measurement.

Sample preparation and laboratory analysis

Multivariate Principal component analysis was used to identify factors influencing relationships between variables, (i.e., Hg concentrations in blood, liver, brain, kidney and hair). The total variance of five principal components (PC) was calculated. Factor coordinates of cases were then used as a new variable. Subsequently, the correlation between morphometric parameters (weight and body length) and factor coordinates was assessed.

The difference in factor coordinates between males and females and between age categories was also investigated. Two age categories were distinguished, adults and immatures (including all juveniles and subadults). Normal distribution was evaluated by the Shapiro-Wilk test. Because the groups did not have a normal distribution, comparisons between two independent groups were made using a non-parametric test (the Mann-Whitney U test).

Based on seasonality, 6 seasonal categories were created (February, March - early spring; April, May, June – spring/summer; July, August - summer; September, October - autumn; November - late autumn; December, January - winter). Before comparing the effect of seasonality, homogeneity of variances was evaluated by Levene's test. When variances did

not differ, ANOVA was applied to assess the seasonal differences. In cases of significant variance of Levene's test (different variances), an alternative Welch F test for unequal variances was used. Different couples were detected by Tukey HSD (honestly significant difference) test.

The threshold for significance of correlations and for two/multiple sample tests was $p \leq 0.05$. The Statistica 7.0 software was applied to calculate the factor coordinates of variables and cases and to detect correlations. Other analyses (Shapiro-Wilk normality test, Levene's test for homogeneity of variances, ANOVA and Welch F test, Mann-Whitney U test, Tukey HSD test) were calculated by PAST4.03.

Results

Full data of mercury (Hg) concentrations in blood, hair, liver, brain and kidney were obtained from 102 mice, and all adults and immatures were included together. These data were used to determine the effect of principal components that influence the concentration of Hg. The coordinates of the principal components based on correlations are presented in Table 1.

PC 1 is a unilinear vector, affecting mercury intoxication of all organs investigated, but acting on individual organs with different intensities. It is least pronounced in the blood and most pronounced in the kidney and liver. This means that if the Hg concentration in the blood increases 3.486-fold under the influence of this factor, the Hg concentration in the kidneys increases 8.515-fold at the same time, and in the liver it rises to 8.962-fold. PC 1 contributes the most to the total variance, resulting from the synchronous and antagonistic effects of all five factors, and represents 45.1% of the total variance.

PC 2 acts independently of the first factor and determines the toxification level in blood. It acts on the blood and liver in a negative direction, and on hair, brain and kidneys in a positive direction. This means that the factor causes an increase in blood and liver concentrations while decreasing brain, kidney and hair concentrations, and vice versa; if blood and liver concentrations decrease, brain, hair and kidney concentrations increase. It accounts for 19.2% of the total variability.

PC 3 affects hair, which is the only variable on which it acts in a negative direction.

PC 4 reflects Hg deposition in the brain, without significant involvement of other organs.

PC 5 acts with nearly equal intensity on the liver and kidneys, but antagonistically.

The effect of morphometric variables, sex, age, and season on the principal components

The effect of morphometric variables, including sex, age, and season on the five strongest principal components was tested. Results (p-values) are presented in Table 2.

The absence of a significant correlation between the coordinates of all five factors and body weight data indicates that one of the factors influencing mercury contamination in mice is not body weight. However, there was a significant negative correlation ($r = -0.40$) between body length and the coordinates of PC 3. This means that PC 3 causes higher concentrations in the brain and lower concentrations in hair in smaller mice and causes lower concentrations in the brain and higher concentrations in hair in larger mice.

An interaction between the PCA factors and sex was not demonstrated, nor was the effect of age. No differences between sexes or age groups were detected.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
Blood	-0.3486	-0.8903	0.0508	0.2852	-0.0443
Hair	-0.5346	0.1403	-0.7927	0.2570	0.0091
Liver	-0.8962	-0.0173	0.1453	-0.2446	0.3400
Brain	-0.5653	0.3830	0.4097	0.6021	-0.0587
Kidney	-0.8515	0.0403	0.0520	-0.4204	-0.3065
% Total variance	45.1	19.2	16.5	14.9	4.3

Table 1. Factor coordinates of the variables, based on correlations, and percent of variance associated with the components.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	test
Weight	0.7429	0.5188	0.2599	0.8389	0.7225	Correlation
Body length	0.1461	0.6491	0.0238	0.9283	0.3421	Correlation
Sex	0.8413	0.5434	0.4392	0.3842	0.5073	M-W
Age	0.3406	0.9594	0,5426	0.9157	0.1345	M-W
Season	0.7743	0.0104*	0.3508	0.0128*	0.0464	ANOVA

Table 2. p-values of relationships between variables and coordinates of cases on factors. * refers to the use of Welch F test in cases of unequal variances. Values in bold refer to significant correlations/differences.

PC 1 coordinates of samples showed no seasonal variation (Fig. 1), and no significantly different seasonal pairs were detected.

Comparison of coordinates of PC 2 across seasonal periods (Fig. 2) indicated that animals are generally intoxicated by mercury in equal amounts throughout the year, except for summer and late autumn. The high PC 2 values of coordinates of samples in the summer contribute to low blood Hg intoxication values in the summer, and the low factor coordinate values in the late autumn result in elevated blood Hg concentrations during this period. High values of factor coordinates of cases during summer were significantly different from the values obtained for late autumn.

The seasonal pattern of the effect of PC 3 (Fig. 3) shows high values of coordinates in autumn and early spring, resulting in low values of Hg contamination in hair. In both following seasons (spring/summer, late autumn) there is a sharp decline, which means that contamination levels in hair are increasing.

PC 4 acts primarily on Hg concentrations in the brain in a positive direction and in the kidneys in a negative direction. Low values for these factors in early spring and spring/summer periods (Fig. 4) account for low levels of brain Hg intoxication during this period. In summer there is a sharp increase in concentration, caused by PC 4. In the other periods there is a slight decrease.

PC 5 causes elevated levels of contamination in kidneys and low concentrations in the liver during early spring and causes high levels of contamination in the liver and low levels of mercury accumulation in the kidneys during other seasons (Fig. 5).

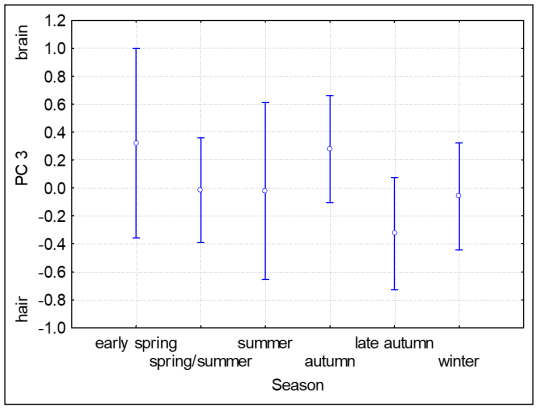

Fig. 3. Factor coordinates of PC 3 in different seasons. Vertical bars denote 95% confidence intervals. There are no statistically different couples (Tukey HSD test).

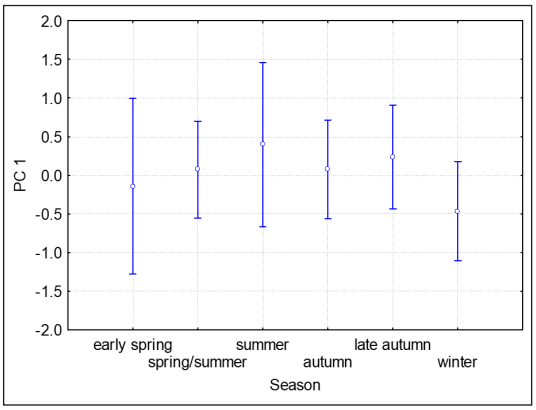

Fig. 1. Factor coordinates of PC 1 in different seasons. Vertical bars denote 95% confidence intervals. There are no statistically different couples (Tukey HSD test).

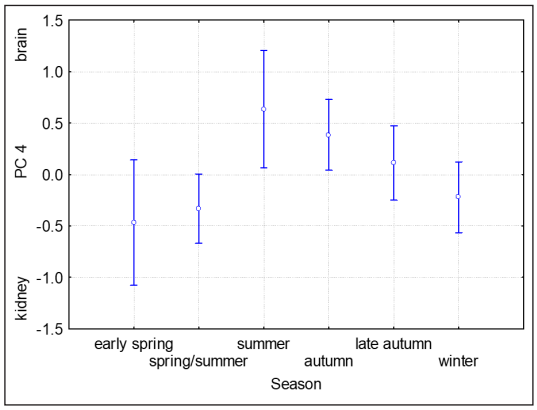

Fig. 4. Factor coordinates of PC 4 in different seasons. Vertical bars denote 95% confidence intervals. Statistically different couple: spring/summer-autumn (Tukey HSD test). See also Table 2.

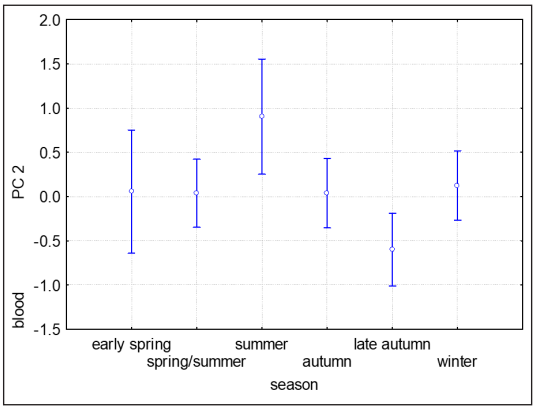

Fig. 2. Factor coordinates of samples (PC 2) in different seasons. Vertical bars denote 95% confidence intervals. Statistically different couple: summer-late autumn (Tukey HSD test). See also Table 2.

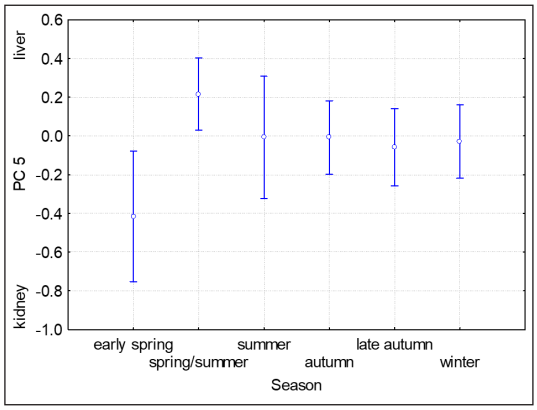

Fig. 5. Factor coordinates of PC 5 in different seasons. Vertical bars denote 95% confidence intervals. Statistically different couple: early spring-spring/summer (Tukey HSD test).

Discussion

Principal component 1

PC 1 acts on all variables in a negative direction. No significant correlation with morphometrics or differences between sex, age or seasonal groups were detected. Therefore, this component cannot be explained exogenously or endogenously (age, sex). This factor affects Hg concentrations in the liver and kidneys, organs where Hg and other contaminants are commonly deposited (Dainowski *et al.* 2015, Antonova *et al.* 2017).

Principal component 2

PC 2 indicates absorption of mercury into blood, regardless of age or sex. The seasonal pattern of this negatively acting factor indicates the lowest values of factor coordinates in late autumn. When comparing the seasonal changes in blood Hg concentrations, it is evident that the highest values of Hg in blood were measured during spring and autumn (Fig. 6). The opposite trend is observed in the summer – Hg values in blood were the lowest. This factor mostly explains low Hg levels in blood during summer months and increase in autumn. However, the body reacts to this sudden influx, because in winter, the amount of Hg in blood decreases, and the high variability observed in autumn months also decreases.

Hg concentrations in blood reflect a short-term state (Yates *et al.* 2014), and unstable and fluctuating levels of Hg in blood are influenced by recent dietary Hg uptake. Because of this, PC 2 may be related to food consumption. Wood mice and yellow-necked mice are considered omnivores. They have a diverse diet with a prevalence of seeds, if their habitat and the season allow (Watts 1968, Green 1979, Montgomery and Montgomery 1990, Zubaid and Gorman 1991, Gorman *et al.* 1993, Rogers and Gorman 1995, Abt and Bock 1998). However, when living in a habitat with a lack seed in production, they can compensate for this deficiency by consuming other plant food (Rogers and Gorman 1995) or animal food in higher quantities (Zubaid and Gorman 1991, Gorman *et al.* 1993). Depending on seasonal availability, mice can adapt to a temporary deficiency of their main food source in their usual woodland habitat. Tree seeds are predominant in the diet of *Apodemus* mice in the autumn and early winter, due to seed abundance and availability. Fewer seeds were found in the stomach contents of mice during spring and early summer. At this time of year, seed resources from the previous autumn begin to be run out, and vegetation has not yet produced additional seeds (Montgomery and Montgomery 1990). Thus when mice lack this main food component, they seek an alternative and consume more animal food. A diet of animal origin is more prevalent in spring and early summer, and is predominated by larvae, and adult insects (Montgomery and Montgomery 1990) such as beetles. Diptera and Lepidoptera, predominate, followed by less abundant centipedes and sporadically molluscs. Mice may also scavenge vertebrate flesh. In the spring, when tree dwelling caterpillars leave the trees to pupate on the ground, those larvae can serve as a reliable source of food for mice (Watts 1968).

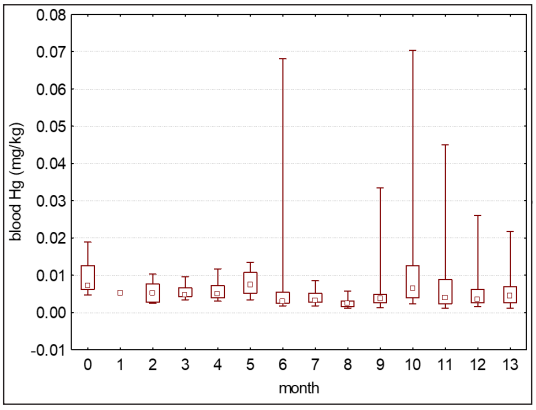

Fig. 6. Concentrations of Hg in mouse blood within season. 0th month is December 2020, 1-12 are January-December 2021 and 13th is January 2022 (Square: Median; Box: 25%-75% Percentiles; Whisker: Min-Max).

Because of the biomagnification of mercury in the food chain, we would expect this high consumption of meat to yield high Hg levels in blood during spring and summer. Therefore, the abundance of a preferred food source (tree seeds) in autumn assumes a decrease of animal food intake and a decrease in blood Hg levels. However, the results we observed yielded the opposite. The mice contain the lowest concentrations of mercury in summer.

One explanation is that more contaminated food is consumed in autumn due to higher moisture and humidity. It is generally accepted that in aquatic environments, animal bodies are more burdened by Hg, because the aquatic environment is more capable of methylation of inorganic Hg (Jitaru and Adams 2004), resulting in persistence of MeHg in animal bodies. In semiaquatic habitats such as floodplains or marshes (Peterson *et al.* 2021), higher Hg levels were found in meso-predator animals whose home range contains wetter areas. Humidity and moisture can increase Hg levels in animal tissues spatially and temporally. Higher concentrations of Hg were found in snow vole (*Chionomys nivalis*) tails during months with more precipitation (Martinková *et al.* 2019), however, in alpine ecosystems, where snow voles were collected, spring was a wetter period than autumn. The climate of the temperate mid-altitude region of Považská Bystrica (including the sampling site) is characterized by hot and dry summers (own observation), which could theoretically explain low summer Hg values.

Another explanation is mycophagy. Unlike plants, which do not accumulate Hg from the soil in high concentrations (Tomiyasu *et al.* 2005), fungi are able to accumulate Hg from the soil at several times higher concentrations than is in the soil (Alonso *et al.* 2000, Falandysz *et al.* 2003). According to Watts (1968), *Apodemus* mice eat the most fungi in the summer (15%) and November (7%). According to Hansson (1971), mosses and fungi were evenly distributed in mouse food throughout the year. The published studies do not refer to an increased

fungi consumption in the autumn season, so mycophagy does not seem to be a sufficient explanation for increased Hg concentrations during the autumn months. But the abundance of mushrooms can be site-specific, and fruiting bodies of edible mushrooms were abundant in some trapping places during autumn 2021 (own observations).

A third explanation is the impact of proteins from an animal-based diet. It was found that laboratory mice fed by a low-protein diet had more Hg in their organs than mice in the control group, fed by an average-protein diet. Additionally, the first group of mice excreted less Hg through urine, though faecal excretion was the same in both groups (Adachi *et al.* 1992). This finding indicates that quantity of protein in diet may have an important effect on animal health and Hg storage in the body and excretion. Animal food can therefore cause two antagonistic effects, higher accumulation of contaminants due to biomagnification, as well as increased excretion from the body.

Finally, seasonal changes in gut microbiota may have an influence on Hg concentrations. Intestinal microbes are an effective barrier preventing MeHg from entering the bloodstream from the intestine (Lapanje *et al.* 2008) as MeHg demethylation has a decisive effect on Hg excretion rates (Rowland *et al.* 1984). As mice can temporarily adapt to different available food sources, there can be observable shifts in gut microbial communities (Maurice *et al.* 2015), depending on type of currently favorable food (plant/animal). It was found that cultures of Lactobacillus isolated from rat intestine can degrade MeHg into volatile Hg0 (Li *et al.* 2019), which is absorbed by the gastrointestinal tract in negligible quantities (Gochfeld 2003). Lactobacillus microbes are more prevalent in microbiota of *A. sylvaticus* during spring but are less prevalent in autumn (Maurice *et al.* 2015). The presence of demethylating intestine microbes in spring can theoretically decrease the accumulation of Hg originating from animal food. A question that emerges, is whether the presence of demethylating microbes is an evolutionary adaptation against higher Hg contamination originating from more contaminated food sources. However, the trade-off does not allow the same defensive mechanism during seasons when less contaminated food items are abundant and consumed. As such, Hg from less contaminated food accumulates in a season when the body is less protected by demethylating microbes.

Principal component 3

PC 3 reflected high contamination levels in hair during late autumn. The antagonistic relationship between PC3 and the amount of mercury in hair and other organs (especially the brain), implies a decrease of mercury in soft tissues during the period of increase in hair. This may imply synchronisation of mercury displacement from organs during seasonal coat exchange.

Given that mammalian hair is an inert tissue (Beltran *et al.* 2018) there should be no changes in the amount of Hg present in hair during the existence of the whole coat, and a possible change would only occur if the whole coat is replaced by a new one (i.e., shedding). However, during the season, there were slight changes observed (Fig. 7). In winter, and at the onset of spring, Hg concentration values were low, but between the end of spring and throughout summer, a gradual increase in concentration was observed. In October, there was a decrease followed by a subsequent increase.

Peterson *et al.* (2021) noted the opposite situation with decreasing Hg concentrations in hair of animals that were captured twice during the season between winter and early summer. This finding is explained by the abrasion of the guard hairs when the distal part of the hair is lost. In this area of hair, Hg is most concentrated, and its concentration decreases toward the base of the hair. There are two hypotheses that explain why most the concentrated amount of Hg is found in the distal end of the hair. One of them is endogenous theory. During hair growth, the distal end of the hair forms first, when the contaminant in blood is in the highest concentration, so that the largest proportion of the contaminant is concentrated in the newly formed hair. As hair continues to grow, the concentration of Hg in the blood decreases as it is stored and deposited into the emerging keratin structures. Therefore, the basal section no longer contains the high concentrations that were present at the initiation of hair growth (Peterson *et al.* 2021). Sobańska (2005) explains this through an exogenous theory, and claims that the distal part of the hair is in the contact with the environment for a longer time period, and thus absorbs the most Hg from the environment.

The observed increase in Hg concentration during summer and late autumn supports both the endogenous and exogenous hypotheses of hair Hg deposition. The newly formed coat contains only endogenously deposited mercury, metabolically degraded from the blood. High autumn Hg levels in hair are to some extent caused by high blood Hg levels during this season (see PC2), and after the direct contact with the environment, hair is enriched with exogenous mercury.

PC 3 is more influential during early summer and late autumn, in moulting and post-moulting periods, whereas later, its effect is hindered by

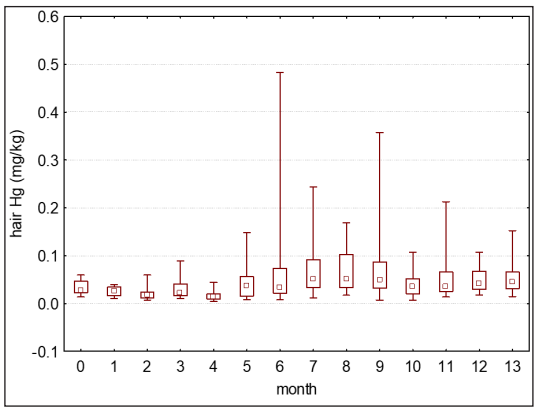

Fig. 7. Concentrations of Hg in mouse hair within season. 0th month is December 2020, 1-12 are January-December 2021 and 13th is January 2022 (Square: Median; Box: 25%-75% Percentiles; Whisker: Min-Max).

the carrying capacity of hair. Small microparticles can accumulate by adhesion or under keratin scales in hair, but this increase in concentration is limited. Additionally, there is the possibility of hair abrasion (Peterson *et al.* 2021), whereby hair sheds some of the concentrated exogenous and endogenous Hg, and the increase in Hg concentration caused by deposition from the environment is slowed. If an abrasion is present, it may represent the participation of both antagonistic factors (exogenous deposition and abrasion), which were not recognized by PCA. The PCA likely combined them into a single factor. Deposition from the environment has a stronger effect in the initial phases of fur duration, and abrasion, occurring later in the cycle, mitigates the effect of exogenous enrichment. Continual decrease in hair Hg concentrations can indicate that abrasion has a stronger effect than exogenous deposition during the months of this decline. An increase in factor coordinates between late autumn and summer and the repetition of this trend during the other half of the year indicates initial rapid toxification of hair and a subsequent slow degradation. In the pre-moulting and moulting seasons, the influence of exogenous deposition is minimized by the exhausted carrying capacity of hair to accumulate exogenous Hg and by subsequent fur exchange cycle.

The hair in this experiment was not washed, while Peterson *et al.* (2021) cleaned sample hair. Thus, there is no clear and precise data on exact concentrations found directly in the hair, though the seasonal pattern provides information about the concentration ratio among months. Sobańska (2005) compares concentrations in washed and unwashed wild boar hair, where washed hair was cleaned using deionized this water. A very strong correlation ($r = 0.99$; $p < 0.05$) reflects the effectiveness of this method, but does not show the delta in how much Hg is deposited from the environment and withstands cleaning. Hair sampling throughout the season did not show significant differences in the concentration of washed and unwashed hair of a wild boar kept in the zoo, although there was a slight indication of changes.

The theory of exogenous deposition of Hg in hair also corresponds to the findings of Gerstenberger *et al.* (2006). Their research was not focused on seasonality, but on a comparison of rodent species living in desert habitats. It has been found that rodents dwelling in burrows have higher Hg concentrations in their hair than species living in above-ground niches, even in the case of animals that have been captured in the same or similar habitat. Liver concentrations were not impacted by whether the animal lived in a burrow or on the surface. *A. flavicollis* and *A. sylvaticus* dig their own burrows, or occupy burrows created by moles (own observation). Gerstenberger *et al.* (2006) also argued that leaf litter prevents direct contact with soil. On the other hand, plant tissues, especially roots and leaves, may also contain trace amounts of Hg. However, their origin is different. Little Hg enters the plant body through the root system. Concentrations in the roots do not reach the same concentrations as in the surrounding soil (Tomiyasu *et al.* 2005), and Hg travels into the above-ground organs of plants in even smaller quantities, as the root system stabilizes absorbed Hg from soil. However, it is also possible to find Hg in the leaves. The main source of Hg in the leaves is either soil vapor (Patra and Sharma 2000) or atmospheric Hg transported from a remote source (Jiskra *et al.* 2018) that enters the humus layer and the soil by litterfall. As low concentrations in all animal tissues indicate an unpolluted site, significant deposition from the atmosphere is not expected, so soil is likely the main source of Hg at the trapping site. Therefore, the hypothesis that soil contact may also affect and increase Hg concentrations in mammalian hair, and be, in addition to bioaccumulation in the food chain, another decisive factor influencing Hg concentration, seems to be based on truth. It is therefore likely that high initial concentration in the newly formed coat, mainly collected endogenously, may be additionally enriched with exogenous mercury from the soil, and thus, the total amount of Hg increases as the fur persists. Small microparticles of soil or dust can accumulate under cuticular hair scales, representing the significant effect of exogenous deposition, which supplements more stable endogenous deposition.

There was also a negative correlation of the factor with body length (Table 2). This negative correlation might result in more Hg in the brain during autumn and early spring in shorter animals, and simultaneously in more Hg in hair during spring/summer and late autumn in longer animals.

Principal component 4

PC 4 acts primarily on the brain in a positive direction. When comparing the seasonal effects of the component, the coordinates of samples of the factor reach the highest numbers in the summer, then decrease in every other season until they reach a minimum during the early spring. Therefore PC4 has the strongest effect in the summer, followed by a lower effect in autumn and late autumn. This factor is the main cause of unexpected high Hg concentration values in the brain during July and August (Fig. 8).

An explanation may be that Hg in the brain originates from inorganic elementary mercury found

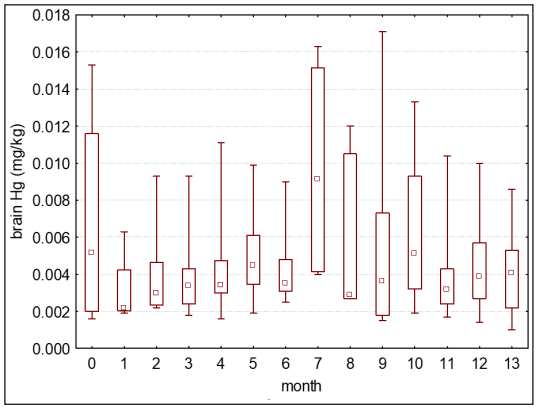

Fig. 8. Concentrations of Hg in mouse brain within season. 0th month is December 2020, 1-12 are January-December 2021 and 13th is January 2022 (Square: Median; Box: 25%-75% Percentiles; Whisker: Min-Max).

in soil. Hg in its elementary form enters the body primarily through inhalation (Gochfeld 2003). It undergoes oxidation in the lungs, but some fraction remains unoxidized and enters the brain through the blood-brain barrier (Warfvinge *et al.* 1992, Chételat *et al.* 2020). Physiologically, increased brain Hg accumulation after exposure to elementary Hg is related to its solubility in lipids, and subsequent oxidation in the brain into its ionic form, Hg^{2+} (Aschner and Aschner 1990). It is likely that elevated temperatures and low rainfall in summer (own observation) caused increased soil dustiness. As burrow-dwelling animals, mice are in direct contact with the soil, which may have increased inhalation of dust containing mercury in its elementary form.

Principal component 5

PC 5 affects primarily the liver and kidneys, organs where mercury accumulates to the greatest extent. In contrast to PC 1, the strongest acting factor, this factor has an antagonistic effect on both organs. Both organs play a role in detoxification, but there are differences in the form of accumulated mercury present in each organ. While kidneys are the target organ for inorganic Hg deposition (Pokorny and Ribarič-Lasnik 2002), the liver mainly exhibits concentrations of the organic methylated form (Kalisinska *et al.* 2021). Therefore, the detoxication process in both organs is likely not co-dependent.

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