



Mercury accumulation and biomagnification in the barn owl (*Tyto alba*) food chain

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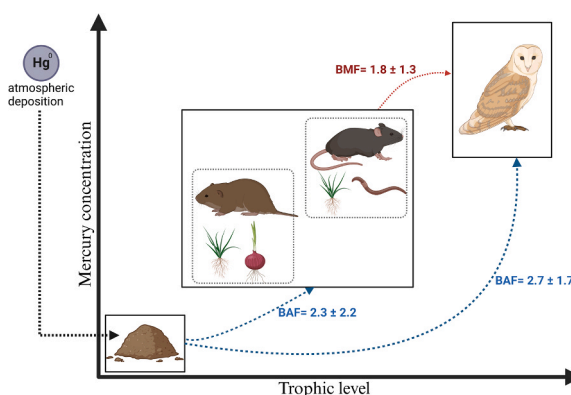
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HIGHLIGHTS

- Mercury (Hg) biomagnifies in the barn owl food chain: highest concentrations in feathers > fur > soil.
- Spatial analysis revealed regional Hg variations and identified local and transboundary pollution sources to top predators.
- The diet of barn owls, feeding omnivorous prey, significantly affects Hg accumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) accumulation and biomagnification in the barn owl (*Tyto alba*) food chain were investigated using bioindicator samples from three trophic levels: (1) soil and moss (atmospheric deposition indicators), (2) small mammal fur from regurgitated pellets (herbivores and omnivores), and (3) barn owl down feathers (apex predators). Spatial analysis identified regional Hg variation in soil, fur and feathers. Statistical models explored the effects of proximity to water bodies, wetlands and nearby pollution sources. The highest total Hg (THg) concentrations were found in feathers ($170 \pm 160 \mu\text{g kg}^{-1}$, $n = 246$) and fur in regurgitated pellets ($150 \pm 200 \mu\text{g kg}^{-1}$, $n = 150$), followed by soil ($63 \pm 17 \mu\text{g kg}^{-1}$, $n = 63$). Bioaccumulation factors were 2.3 (soil to fur) and 2.7 (soil to feather). Biomagnification factor from fur to feathers was 1.8. Methyl Hg (MeHg), measured in a subset of samples, was $120 \pm 130 \mu\text{g kg}^{-1}$ in fur ($n = 29$) and $150 \pm 98 \mu\text{g kg}^{-1}$ in feathers ($n = 42$), with 75–97 % of THg in feathers as MeHg. Prey composition significantly influenced fur THg levels,

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with higher concentrations in diets with omnivorous prey (*Apodemus flavicollis*) compared to herbivorous prey (*Microtus arvalis*). These findings highlight the importance of diet in Hg monitoring and biomagnification studies.

1. Introduction

Mercury (Hg) is a pollutant of concern due to its toxicity and widespread occurrence in the environment. Its ability to bioaccumulate and biomagnify in food chains poses an ecological risk [61,70]. Globally, Hg pollution is related to anthropogenic activities such as artisanal and small-scale gold mining (ASGM) as the key emission source, coal and waste combustion, industrial processes including cement manufacturing and metal smelting [100]. These activities release elemental Hg (Hg⁰) into the atmosphere, which is translocated and deposited in land and water bodies, even in remote regions such as the Arctic [7]. Once deposited, atmospheric Hg undergoes processes such as oxidation-reduction and microbial biomethylation, transforming it into organic forms such as methylmercury (MeHg) [49]. MeHg is the toxic form of Hg, that biomagnifies in food chains with increasing trophic levels, posing a threat to the top predators [105,85,98]. The biomethylation of Hg⁰ and inorganic mercury (Hg²⁺) usually occurs under anoxic conditions, such as in water bodies, sediments, wetlands and waterlogged soils. While most methylation occurs in aquatic environments, recent studies have begun to explore the transfer of Hg from aquatic to terrestrial ecosystems [56] and the role of habitat biogeochemistry [50], which remains an area of growing interest. The interplay of biochemical, physical, and ecological processes makes Hg transformation, accumulation and biomagnification more complex, leading to spatial variation and variations across organisms [30]. This complexity creates a gap in our understanding on how biological factors of organisms like diet preferences and age combined with environmental factors like their habitat, influence the accumulation from the environment, movement through food webs and distribution across the geography.

For a comprehensive understanding of Hg's effects on wildlife, especially top predators and endangered species, it is crucial to monitor Hg concentrations in environmental matrices such as soil and organism tissues, identify local hotspots, and investigate spatial distribution [11]. Despite its importance, few studies have integrated spatial distribution patterns with biological and environmental factors [53,71,80]. This knowledge gap is critical in predicting the long-term ecological impacts and assessing the effectiveness of the Minamata convention, a global treaty that aims to reduce Hg pollution and monitor environmental and health impacts [69].

The negative effects of Hg on the health of organisms in both terrestrial and marine environments are well-documented [25,98]. Several studies have highlighted Hg's effects on bird survival and reproduction [110,22,64], particularly for species at higher trophic levels [105,19,35]. Least-invasive samples, such as bird feathers and mammal fur, are commonly used to monitor environmental pollution [57,81,97]. MeHg is sequestered into these keratin structures by binding with sulfur groups [21,47,66,83], with accumulation linked to blood circulation during feather and fur growth [14,16,43]. Feather molting and fur shedding help the organisms in detoxification [34,58,101]. Down feathers are often recommended over adult feathers for Hg analysis due to lower variability in THg concentrations and their strong correlation with juvenile body burdens [3,4]. Additionally, Hg in fur significantly correlates with Hg levels in other tissues [23,44,109].

It is a widely accepted practice in contaminant monitoring to use an indicator species to assess the presence and distribution of an environmental contaminant. The chemical concentration measured in higher trophic level consumer often represents the environmental contamination levels within the consumer's home-range. This study focuses on the barn owl food chain in Switzerland. Barn owls are non-migratory birds and are at the top of their food chain. They have a simple food web,

consisting mainly of small mammals such as voles and mice [89]. Due to their position in the food chain, barn owls serve as useful bioindicators, although the variability in small mammal diets, which can include both plants and insects, introduces complexity into the food web [42,89]. Barn owl juveniles take their first flight at around 55 days of age [90]. During the period from hatching to flight, exposure to Hg occurs mainly via food brought by the parents through hunting in the vicinity of the nest. Down feathers from juveniles can therefore be valuable indicators of local Hg and MeHg exposure. In Switzerland, the primary sources of atmospheric Hg include fuel combustion (petroleum), waste incineration for power generation, cement and steel production, Hg recycling plants and long-range transboundary transport [41,8]. Additional sources are re-emission from legacy pollution sites that have reported elevated soil Hg levels [86,76]. In this study, we quantified THg in down feathers from juvenile barn owls, in fur collected from regurgitated pellets found in the nests, and in composite soil samples collected around the nests in the barn owls' primary hunting grounds. We also quantified MeHg in the down feathers and fur samples to determine the fraction of MeHg in THg. Furthermore, we assessed the spatial distribution of THg in soil, fur and feathers to identify potential hot spots and outliers. We examined environmental (local and diffuse Hg sources) and biological factors (age and diet composition) to understand their contribution to Hg accumulation in these matrices. We expect Hg to accumulate in the tissues of organisms from the environment and Hg biomagnifies within the barn owl's food chain through their diet, with higher proportions of omnivorous prey leading to greater Hg accumulation in feathers [28,10].

2. Methods

2.1. Study sites

Down feathers and regurgitated pellets from nestling barn owls at 67 breeding sites in four regions of Switzerland (Central Plateau, Jura, Northern Plateau and Southern Plateau that includes three sites from the Valais situated at southern-most part) were collected, and soil samples were taken within a 1.5 km radius (Fig. 1). The barn owl breeding sites sampled in our study were located at altitudes between 379 and 835 m above sea level and are monitored as part of an existing integrated population monitoring program of the Swiss Ornithological Institute [94].

Given the opportunistic nature of the sampling, we divided the sample sites into four regions: Central Plateau, Jura, Northern Plateau and Southern Plateau. The Central, Northern and Southern Plateau are parts of the Swiss Plateau (in biogeographical division terms used in Switzerland). The Jura and Swiss Plateau differ in geography, land use, industrial emissions and urbanization. The Jura region is primarily dominated by agricultural and forested areas, though settlement and urbanization are gradually increasing [37]. As the first major mountain ridge encountered by the weather systems moving eastward from the Atlantic Ocean and the orographic effect, the Jura region experiences higher precipitation than the Swiss Plateau.

In contrast, the Swiss Plateau is the most densely populated area of Switzerland. The wind conditions are generally weak, with prevailing winds originating from the west or northeast, especially in valley regions [72]. Industrial emissions are significantly higher in the plateau compared to the Jura, with major industrial sites concentrated near large urban centers such as Zurich and Winterthur [40].

Additionally, the Southern and Central Plateau have known legacy Hg release sites and are particularly relevant for assessing the impact of anthropogenic activities and subsequent influence in the terrestrial food

chain.

2.2. Sampling and sample preparation procedure

2.2.1. Soil

We collected soil samples from four meadows within a radius of 1.5 km around the nests, approximately the size of the barn owl hunting area [6,87]. All samples were kept at 4 °C and later transferred to −20 °C in the laboratory within 24 h. We freeze-dried the soil samples and sieved them (<2 mm). We milled them in a planetary ball mill (PM 100, Retsch) using agate balls and stored them at −20 °C until further analysis. Detailed procedures for soil sample collection and preparation can be found in the [Supplementary Information](#) (SI).

2.2.2. Prey tissues

To measure Hg in the fur of small mammals eaten by barn owls (prey), we randomly collected five to ten regurgitated pellets from each nest (see SI). We dissected 271 pellets, separating the fur and bones from each pellet. Using cranial characteristics [104], we identified the species to which the skulls belonged (for example: voles, shrews). The fur underwent a series of washing steps to remove any exogenous contaminants. We removed visible large and some small bones from washed fur samples, then freeze-dried and cryomilled the fur for homogenization. The resulting homogenized fur samples (in powder form) were stored at −20 °C until further analysis.

In addition, we analyzed frozen specimens of two primary prey species of the barn owl: common vole (*Microtus arvalis*) (primarily herbivore) [67] and yellow-necked mouse (*Apodemus flavicollis*) (omnivore)

[89] to verify the difference in accumulation of THg levels in the prey tissues based on the prey species' feeding habits. These samples were collected between 2016 and 2017 from one farm in Uettiligen (Canton of Bern). These individual specimens have been stored frozen at −20 °C in the Institute of Ecology and Evolution (IEE) at University of Bern. Necroscopies were performed on partially thawed prey over an ice tray (see SI for details of sample origin). We collected fur, muscle, entire liver, both kidneys, and the entire brain from these specimens. The fur underwent the same cleaning and homogenization process as the fur collected from pellets. The organs were freeze-dried, and the entire organ was analyzed for THg without further homogenization. Detailed procedures for prey tissue collection and preparation can be found in the [Supplementary Information](#).

2.2.3. Down feathers of juvenile barn owls

When the oldest juvenile barn owl in a brood was between 28 and 45 days old, we collected loose down feathers from all juveniles in the brood, aged between 14 and 45 days. In a few cases where the youngest siblings of a brood had incomplete shedding of down feather, we carefully trimmed them above the calamus using scissors cleaned with ethanol. Each juvenile contributed between 0.2 and 1 g of feathers, individually stored in labeled plastic zip-lock bags and kept at −20 °C until further preparation. In the laboratory, down feathers from individual juveniles were washed sequentially to remove exogenous contaminants. Subsequently, all feathers underwent a 48-hour freeze-drying process followed by cryo-milling to achieve homogenization. The resulting homogenized down feather samples (in powder form) were kept at −20 °C until further analysis. Detailed procedures for down

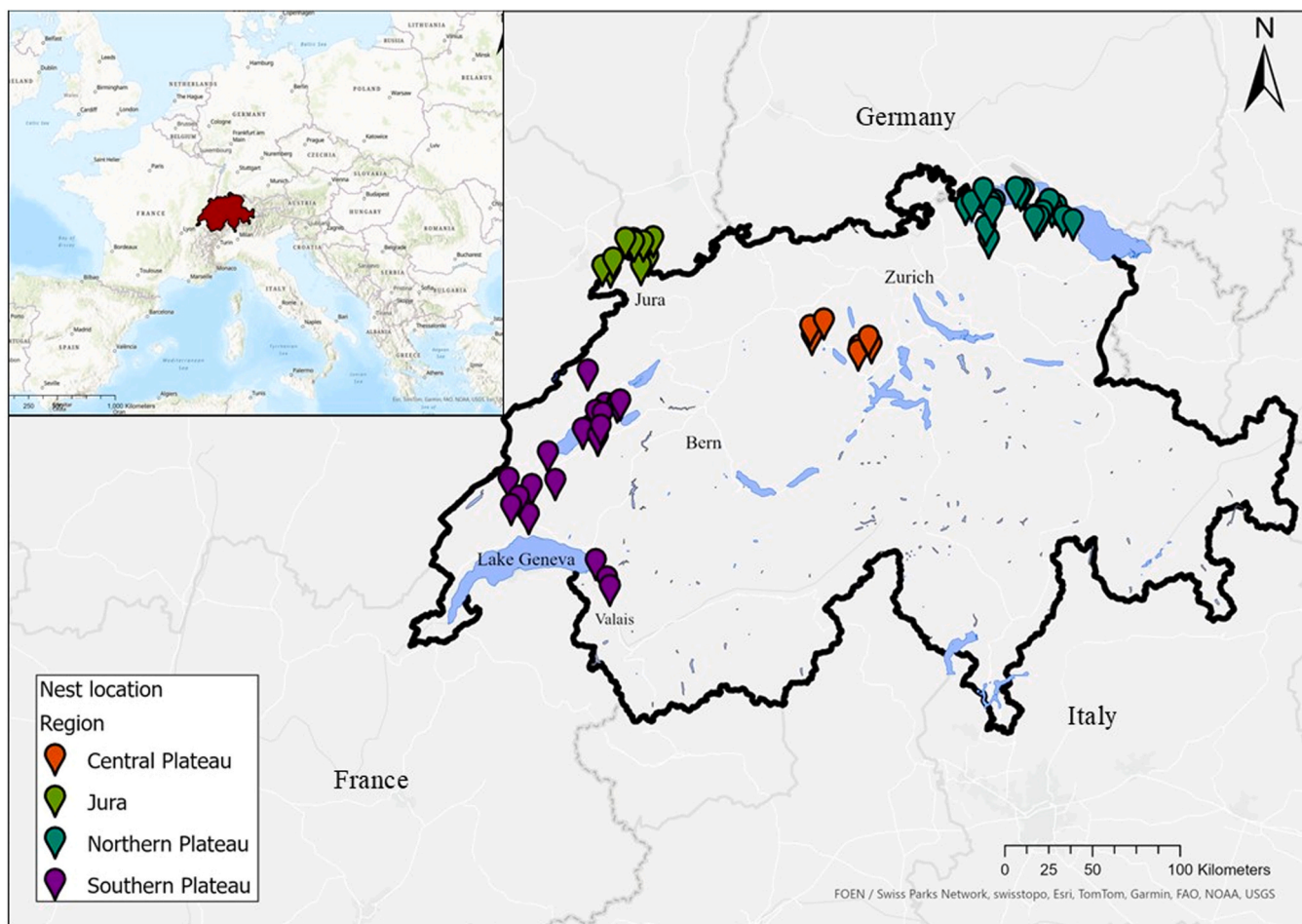


Fig. 1. A map of Europe highlighting Switzerland (top left). In the Switzerland map, nest locations are marked by colored place markers for four regions: Central Plateau, Jura, Northern Plateau and Southern Plateau (including three sites from the Valais, situated at southern-most part).

feathers collection and preparation can be found in the [Supplementary Information](#).

2.3. Mercury analysis

2.3.1. Total mercury (THg)

The THg concentration in various samples (100 mg of homogenized soil, 20 – 50 mg of homogenized prey fur from nests and juvenile feathers, as well as whole freeze-dried prey tissues) was quantified using a Direct Mercury Analyzer (DMA-80 evo, Milestone). The THg concentrations are reported in $\mu\text{g kg}^{-1}$ dry weight (dw) of the sample.

We used Montana II Soil -SRM2711a (National Institute of Standards and Technology-NIST) and loam soil -ERM-CC141 (European Reference Material) as certified reference materials (CRMs) for soil. The recovery mean $\pm$ standard deviation (SD) for Montana II soil and loam soil were $97 \pm 4.5\%$ ($n = 9$) and $92 \pm 4.0\%$ ($n = 12$), respectively. For fur and feather samples, we used human hair ERM DB-001 (Joint Research Center- JRC) as CRM with a recovery of $94 \pm 9.7\%$ ($n = 33$). For the tissues of small mammals, we used mussel tissue NIST 2976 as CRM with recovery of $120 \pm 9\%$ ($n = 9$).

2.3.2. Methylmercury (MeHg)

Batch extractions were performed on subsets of fur and down feather samples for MeHg using aqueous 0.1 % w/v L-cystein.HCl-H₂O (L-cysteine hydrochloride monohydrate, VWR Chemicals) + 0.1 % w/v L-cysteine (L-cysteine, Merck) as extract at pH $\sim$ 2.35 [52]. 50 mg of homogenized sample was extracted with 10 ml of L-cysteine solution, at 80 °C on a hot block for 2 h [92]. The extract was then filtered through 0.22- μm polytetrafluorethylene (PTFE) hydrophilic filters (25 mm, BGB). Hg compounds in 100 μl of filtered extract were separated by reversed phase high-performance liquid chromatography (HPLC 1260 Infinity, Agilent Technologies Inc.) using a C-18 column (Zorbax SB-C18, 4.6 x 150 mm, 5 μm , Agilent Technologies Inc.) and a mobile phase with 98 % v/v of aqueous 0.1 % w/v L-cystein.HCl-H₂O + 0.1 % w/v L-cysteine and 2 % v/v HPLC grade methanol at a flow rate of 1 ml min⁻¹ at room temperature. Hg compounds were detected by coupling an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7700x, Agilent Technologies Inc.) to the HPLC. The LoD in extract was $< 0.08 \mu\text{g L}^{-1}$. For 50 mg sample the LoD was $0.02 \mu\text{g kg}^{-1}$ dry weight of sample. We used human hair reference material NIMD-01 as CRM. The extraction efficiency of the Hg species extraction method was $73 \pm 2.4\%$ (mean $\pm$ SD, $n = 8$). The column recovery was $102.5 \pm 3.0\%$ (mean $\pm$ SD, $n = 8$). The MeHg recovery using the CRM was $96 \pm 5.9\%$ (mean $\pm$ SD, $n = 6$).

Detailed information regarding the extraction and analysis methods for Hg and MeHg can be found in the [Supplementary Information's](#) method section.

2.4. Spatial interpolation and exploration

As a measure of atmospheric Hg (Hg₀) deposition in 2020, we used the THg levels of mosses in Switzerland measured as part of the European Moss Survey [55]. This is the latest available moss data for Switzerland for the sampling period. We used Empirical Bayesian kriging (EBK) (ArcGIS Pro 3.1.0) to account for non-stationarity of data. We predicted moss THg concentrations at unsampled locations and extracted predicted moss THg values at the nest locations. Parameters used in the interpolation method are listed in the [Supplementary Information](#) (Table S 6).

We used spatial join to calculate the distance between the nest sites and proximity to Hg release point sources using Swiss Pollutant Release and Transfer Registry (PRTR) database [40] from 2021 (corresponding to year of sampling), as well as the distance to the closest wetlands [38], large water bodies [39], and urban cores (a minimum size of 50,000 inhabitants) [36]. We applied optimized hotspot analysis to identify statistically significant spatial clusters of high (hot spots) and low (cold

spots) Hg values in soil, fur and feather. Additionally, we used optimized outlier analysis to identify soil, fur and feather Hg values that were significantly different from those of their neighboring nests.

2.5. Statistical analyses

For normal linear model, the function `lm` from the R (version 4.2.2) stats package [82] was used, and for mixed-effect models the function `lmer` from the package `lme4` [13] was used. For both normal linear model and mixed-effects models, we used a Bayesian approach and calculated 95 % credible intervals (denoted as CrI) for parameter estimates, model predictions and derived statistics by drawing 20,000 random samples from the posterior distributions of the model parameters using the `sim` function in the `arm` package [9].

To model the soil THg around nest sites, we used a linear regression model (Model A) with elevation of nest sites, predicted moss THg at nest site, Hg emission value of point source, proximity to point sources, wetlands, large water bodies, urban cores, and regions as covariates.

To model the average fur THg of barn owl prey found in the pellets (hereafter fur THg), we used a linear mixed-effect model (Model B) with soil THg, prey composition with different feeding habits, prey number, predicted moss THg at nest locations and study region as covariates and nest ID as a random intercept.

To model feather THg we built a second linear mixed-effect model (Model C) with age of juvenile barn owls at sampling (in days), the average fur THg per nest (hereafter average fur THg), average soil THg, predicted moss THg at nest locations and study region as covariates and nest ID as a random intercept.

Kruskal-Wallis's test was used to compare soil, fur and feather THg concentrations across the study regions followed by post-hoc pairwise comparisons (Dunn's test with Bonferroni correction). Wilcoxon's test was used to compare tissue THg of *M. arvalis* and *A. flavicollis*. For each prey species, we compared the THg distribution in tissues using Friedman's test.

The bioaccumulation factors (BAF) of THg from soil to the pellet fur and barn owl feathers were calculated by dividing by soil THg respectively. The biomagnification factors (BMF) between predators and their prey in each brood were calculated by dividing the average feather THg in each brood by average fur THg.

3. Results and discussions

3.1. Soil mercury

The average soil THg around barn owl breeding sites ranged from 35 to 118 $\mu\text{g kg}^{-1}$ (mean $\pm$ SE: $63 \pm 17 \mu\text{g kg}^{-1}$; $n = 63$), which is below the Swiss soil guideline value of 500 $\mu\text{g kg}^{-1}$. Our values align with the Swiss National Soil Observation Network (NABO)'s soil THg values reported for 2010 – 2015 ($2.5 - 2684.3 \mu\text{g kg}^{-1}$; $n = 1012$) [48] and the recent Swiss geochemical soil atlas [84]. The mean soil THg in our study exceeded the predicted no effect concentration (PNEC) in soil for terrestrial organisms ($22 \mu\text{g kg}^{-1}$ dw) provided by the registrants of Hg under the REACH regulation (Registration, Evaluation, Authorization and Restriction of Chemicals). However, our values were below the no observed effect concentration (NOEC) for soil microorganisms ($1400 \mu\text{g kg}^{-1}$ dw), soil arthropods ($2700 \mu\text{g kg}^{-1}$ dw) and soil macro-organisms except arthropods ($3700 \mu\text{g kg}^{-1}$ dw) [32]. The soil THg levels found in this study were lower than those measured at hotspots in mining areas and coal-fired power plants in European Union topsoil, which often exceed 400 $\mu\text{g kg}^{-1}$ [11], although the median in this study ($60 \mu\text{g kg}^{-1}$) exceeded the median of 23 $\mu\text{g kg}^{-1}$ reported for European Union topsoil [78].

Soil THg concentrations differed significantly between the study regions (Kruskal-Wallis's test; $p = 0.004$; Fig. 2 A). The highest concentration was observed in the Central Plateau ($75 \pm 16 \mu\text{g kg}^{-1}$, $n = 8$), exceeding those of the Northern Plateau ($68 \pm 17 \mu\text{g kg}^{-1}$,

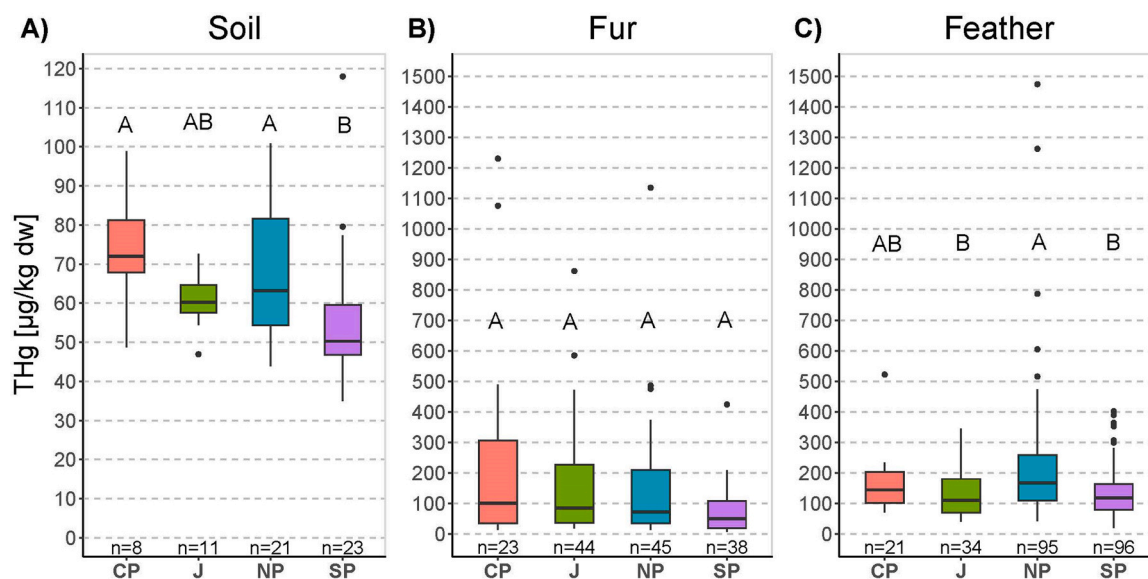


Fig. 2. Box plots represent the distribution of total mercury concentrations (dry weight) in each region for soil (A), fur (B) and feather (C). The regions are Central Plateau (CP), Jura (J), Northern Plateau (NP) and Southern Plateau (SP). The Kruskal Wallis test was used to check statistically significant differences between the study regions, followed by post-hoc pairwise comparisons (Dunn's test with Bonferroni correction). Compact Letter Display (A, B, AB) shows that the total mercury concentrations in regions labeled AB is similar to both A and B, but A and B are still significantly different from each other.

n = 21), the Jura ($61 \pm 7.0 \mu\text{g kg}^{-1}$, n = 11) and the Southern Plateau ($56 \pm 18 \mu\text{g kg}^{-1}$, n = 23). The THg concentration in the regions showed significant variability, implying that the concentrations vary within the regions. Pairwise comparisons showed significant differences between the Northern Plateau and the Southern Plateau ($p = 0.03$), as well as between the Central Plateau and the Southern Plateau ($p = 0.01$). The linear regression model for soil (Table S8, Model A) showed a positive significant correlation with predicted moss THg at the nest site [mean estimate 13.9; 95 % CrI 0.7 – 26.9], which indicates accumulation of atmospheric Hg in both moss and soil. No other covariates significantly influenced the variation in soil THg. Soil properties also play a crucial role in sequestering Hg from the atmosphere. For example, soils with high organic matter content can more effectively bind Hg to thiol groups, thereby promoting Hg immobilization [86]. Similarly, clay particles in the soil enhance the surface area available for the adsorption of atmospheric Hg [75]. Although we did not directly investigate soil properties, our model suggested that atmospheric deposition was a major contributor to Hg accumulation in soils at the nest sites. The correlation between atmospheric deposition and Hg accumulation at nest sites further highlights the significance of moss as a bioindicator of Hg deposition [12,68,93], reflecting the environmental load of Hg in soils.

3.2. Prey tissues

3.2.1. Prey identification

569 skulls from 271 pellets collected from 56 nests were identified by cranial characteristics as 64 % voles (family *Cricetidae*), 24 % *Apodemus* (family *Muridae*), 12 % shrews (family *Soricidae*), and one small bird. This prey species composition was similar to Roulin's [89].

3.2.2. Mercury levels in fur from pellets

The fur THg ranged from 7.1 to 1200 $\mu\text{g kg}^{-1}$, with a mean $\pm$ SD of $150 \pm 200 \mu\text{g kg}^{-1}$ (n = 150 pellets of 52 nests). Fur MeHg was analyzed in a subset of 33 pellets from 150 pellets from 22 nests. Based on skull identification, the species in these 33 pellets were voles (n = 17), *Apodemus* (n = 8), shrew (n = 1) and unidentified (n = 4), with three additional pellets containing mixed prey species (one pellet containing two *Apodemus* and two pellets containing one vole and one

Apodemus). MeHg values in fur from pellets ranged from $< \text{LoD}$ ($0.08 \mu\text{g L}^{-1}$ in the extract) to $390 \mu\text{g kg}^{-1}$ ($120 \pm 130 \mu\text{g kg}^{-1}$; n = 29). The MeHg fraction of the THg measured by DMA-80 ranged from 32 % to 103 % ($76 \pm 19 \%$; n = 29). This fraction was comparable to the MeHg fraction found in human hair (similar keratin structure) in studies from Japan [51], Bolivia, and France [60]. MeHg bound in keratinized tissues are an important pathway of elimination in terrestrial vertebrates [101] and represents significant portion of the total Hg body burden [20].

Fur THg concentrations differed significantly between the study regions (Kruskal-Wallis's test; $p = 0.027$), but pairwise comparisons (Dunn's test with Bonferroni correction) did not show any significant differences (Fig. 2 B). The prey number found in each pellet and prey composition (mixed diet) were significant predictors for fur THg (Table S9, Model B). Nest-level clustering explained 20 % of the variance, while the full model explained 40 % (conditional r^2). Compared to feeding on herbivore species alone, predation on mixed prey species was associated with significantly higher fur Hg levels (Figure S3).

The positive effect of prey number on fur THg levels supports bioaccumulation by prey at higher trophic levels in the food web [59]. In addition, the THg levels in fur were specifically higher in pellets containing mixed prey species than in pellets containing only herbivore prey.

3.2.3. Mercury levels in prey tissues

Investigating Hg levels in prey tissues enables us to confirm that the higher accumulation of Hg in predator's tissues is based on the feeding habits of prey. The correlation between tissue THg concentration varied between the omnivorous *A. flavicollis* and the herbivorous *M. arvalis*. In *M. arvalis*, we did not find significant correlations among the tissue pairs, except between muscle and liver (Spearman $r = 0.66$; $p = 0.03$; n = 11). In *A. flavicollis*, fur THg showed significant correlation with kidney (Spearman $r = 0.83$; $p = 0.005$; n = 10), liver (Spearman $r = 0.83$; $p = 0.005$; n = 10) and muscle (Spearman $r = 0.83$; $p = 0.005$; n = 10). The tissue accumulation also varied between the omnivorous *A. flavicollis* and the herbivorous *M. arvalis* (Fig. 3). The omnivore accumulated significantly higher THg in fur (Wilcoxon's; $p < 0.001$), liver ($p < 0.001$) and muscle ($p < 0.001$). While kidney THg were similar, the brain THg was significantly higher in the herbivore

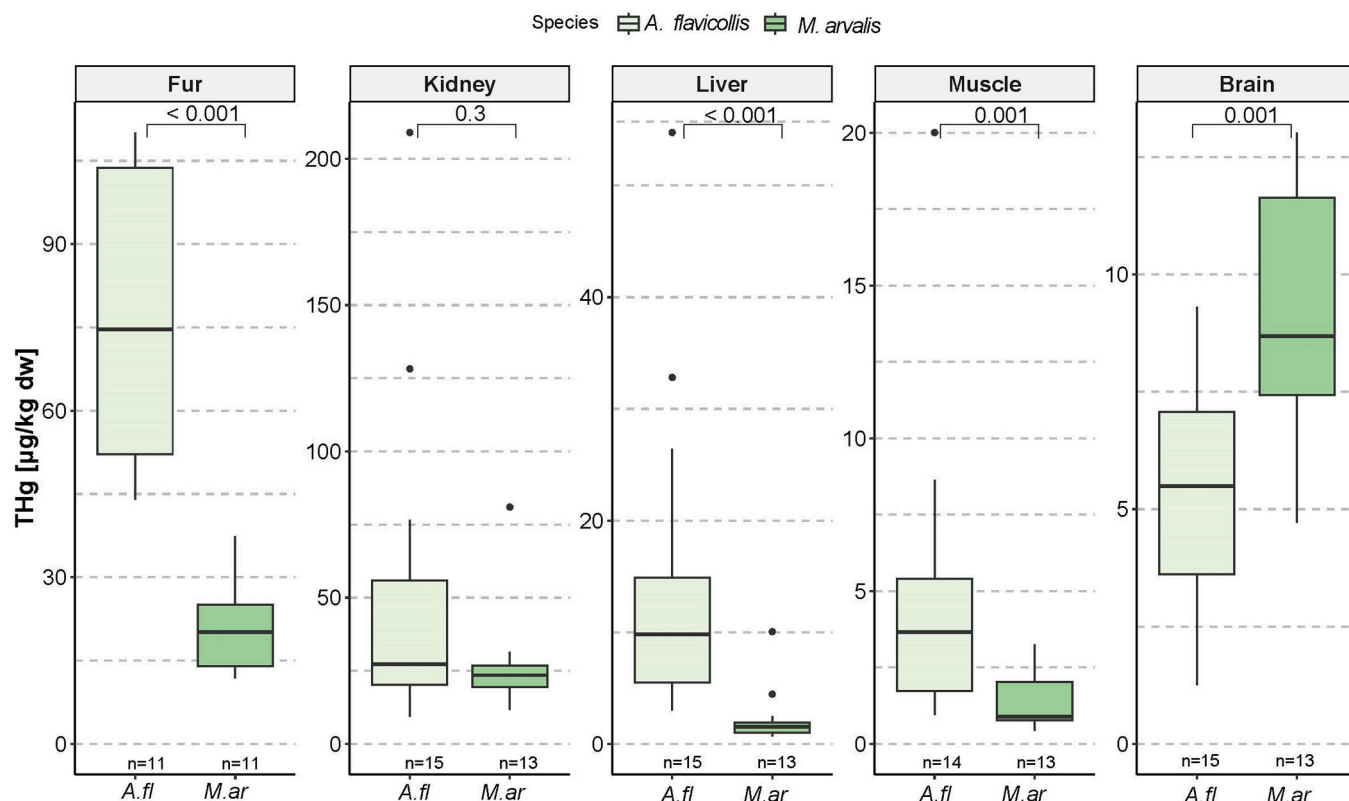


Fig. 3. Total mercury concentrations in tissues (fur, kidney, liver, muscle, and brain) of omnivorous *Apodemus flavicollis* (A. fl) and herbivorous *Microtus arvalis* (M. ar). Wilcoxon's test is used to assess the significant difference between species for each tissue.

($p = 0.001$). Such a difference in metal accumulation has also been observed in studies, where tissues from small mammals that primarily consume insects and carcasses had significantly higher metal concentrations than tissues from voles and other mouse species that primarily consume plants [63,103].

In contrast to our findings, Durkalec et al. [29] reported significantly higher liver THg levels in herbivorous *M. arvalis* compared to omnivorous *A. flavicollis*, with additionally significant higher liver Hg levels in males and a significant positive correlation with body weight for four species of wild terrestrial rodents. We did not find such influence of sex and body weight on the tissue concentrations. Hg accumulates in the brain mainly through exposure to MeHg, which crosses the blood-brain barrier, and through the demethylation of MeHg to Hg, it binds to selenium (Se) forming Hg-Se complexes and resulting in immobilization of Hg in the organism's body [79,102]. We hypothesize that Hg-Se interaction may have influenced the higher accumulation of Hg in the brain of *M. arvalis*. Sakamoto et al. [91] and Liu et al. [62] reported that mice co-exposed to Hg and Se exhibited Hg accumulation in the brain, whereas mice exposed only to Hg did not show any Hg accumulation in their brain. Newland et al. [73] reported that brain Hg of mice exposed to high MeHg increased with time and the increment depended on the exposure to increasing levels of dietary Se intake. It is possible that the dietary intake of bioavailable Se was higher from plants and soil for herbivore than for omnivore, resulting in this difference in tissue distribution.

Fur THg found in the prey species from the specimen collection had lower values compared to the fur THg measured from the pellets. Hg found in all organs of the prey species from the specimen collection was generally low which might be a location specific effect stemming from the origin of the specimen. The THg measured in the liver of *A. flavicollis* was lower than liver values measured in the same species in Slovenia close to thermal power plants ($3 - 173.3 \mu\text{g kg}^{-1} \text{ ww}$; $n = 30$), while the values for *M. arvalis* in our study were similar to another study [5]. These

concentrations are significantly lower than the lethal levels of approximately 30 mg kg^{-1} in the liver and kidneys of mammals [58], and below NOEC of 7 mg kg^{-1} of food in mammals [32].

3.3. Feathers

The individual juvenile down feather THg ranged from 20 to $520 \mu\text{g kg}^{-1}$ ($160 \pm 98 \mu\text{g kg}^{-1}$; $n = 241$) and exceeded the LoD of $0.8 \mu\text{g kg}^{-1}$. We observed significant differences in feather THg among the regions (Kruskal-Wallis's test, $p = 0.002$), with significantly higher feather THg in the NP ($190 \pm 110 \mu\text{g kg}^{-1}$, $n = 90$) than the SP ($130 \pm 83 \mu\text{g kg}^{-1}$, $n = 96$) and Jura ($130 \pm 78 \mu\text{g kg}^{-1}$, $n = 34$), and similar values to the CP ($160 \pm 96 \mu\text{g kg}^{-1}$, $n = 21$) (Fig. 2 C). We did not observe any significant relationships between the feather THg and the age of juveniles as reported in other studies [109,110,46]. These results suggest that THg in down feathers is not influenced by the age of the nestlings. This could be due to differences in how each juvenile absorbs, distributes, and eliminates Hg into their growing feathers, regardless of their age. Conversely, Ackerman et al. [2] reported that feather Hg decreased with age in juvenile waterbirds.

Down feathers likely accumulate lower levels of Hg than body feathers due to their shorter exposure time to Hg in the blood as these feathers form during the early stages of growth and are very small. This is supported by our observations that down feather THg ($20 - 520 \mu\text{g kg}^{-1}$; $n = 241$) was lower than the concentration reported in barn owl body feathers from other studies [24,88]. Moreover, down feathers develop when the juveniles are nest-bound and dependent on their parents for food. The parent's choice of prey could limit the juveniles' Hg exposure through diet. From the linear mixed-effect model (Table S9, Model C), we observed that fur and soil THg were significant predictors of feather THg. Feather THg levels increased with higher fur THg (Figure S3 b), while they decreased with higher soil THg. One possible explanation for the negative effect of soil THg is that Hg in soil

may not be bioavailable to the barn owl food web. Hg deposited from the atmosphere could be sequestered in organic matter or bound in immobile inorganic forms, limiting its bioavailability.

Similarly, the absence of relationship between feather THg and moss THg may suggest that Hg deposited in moss is not readily transferred to higher trophic levels. Additionally, no effects were observed for juvenile age, predicted moss THg, or proximity to point source, urban core, or wetlands.

The results of model C questions the influence of spatial variation in Hg deposition. The lack of significant relationship between Hg deposition and observed feather THg concentrations suggests that regional differences in atmospheric deposition have minimal influence on mercury accumulation, reinforcing the dominant role of diet. A study on Bonelli's eagles from south-western Portugal, which documented Hg concentrations in feathers as a function of the proportion of secondary consumers in the diet [77], supports our results from model B for THg in fur and model C for THg in feathers. This suggests that Hg accumulation in tissues is relatively high when top predators consume more prey from high trophic levels (herbivores vs omnivores) [15,35].

A subset of down feathers of 42 individuals was analyzed for MeHg. The MeHg in juvenile down feathers ranged between 29 and 490 $\mu\text{g kg}^{-1}$ ($150 \pm 98 \mu\text{g kg}^{-1}$; $n = 42$). MeHg and THg of the juvenile down feathers showed a positive correlation (Spearman $\rho = 0.99$, $p < 0.001$). The MeHg fraction of the THg ranged between 75 % and 97 % (84.4 ± 3.7 %; $n = 42$). This aligns with findings in the literature, where MeHg is consistently reported as the dominant Hg form in feathers of both terrestrial birds [106,107] and seabirds [83].

3.4. Bioaccumulation and biomagnification

The bioaccumulation factor (BAF) of THg from soil around the nests to fur in pellets was 2.3 ± 2.2 (0.2–11; $n = 51$), whereas BAF from soil around the nests to feathers in each clutch was 2.7 ± 1.7 (0.6 – 8.0; $n = 56$). The biomagnification factor (BMF) of THg from fur in pellets to feathers in each clutch was 1.8 ± 1.3 (0.2 – 6.0; $n = 45$). An important limitation of our study is the difference in dominant Hg species across matrices. Soil primarily contains inorganic Hg, whereas fur and feathers predominantly contain bioavailable MeHg (75–97 %). Due to this discrepancy, we can only estimate the THg bioaccumulation factor. While our findings confirm the accumulation and transfer of THg within the barn owl food chain, the interpretation of the bioaccumulation and biomagnification factors requires caution. Instead of analyzing stomach contents, as done in other studies [18], we used fur as a proxy for dietary Hg. This is because we used a least-invasive sampling technique and relied on juvenile down feather to measure Hg concentrations. As shown in Fig. 3, Hg accumulation in fur of prey was significantly higher than in other tissues, potentially leading to an overestimation of dietary Hg exposure and an underestimation of biomagnification. Furthermore, Hg eliminated in fur and bound to keratin is less likely to be bioavailable to barn owls than Hg accumulated in muscle or other organs, which could increase the uncertainty of our biomagnification estimates although Hg in fur provides a good estimation of body Hg [31,33]. The relatively low BMF from fur to feathers (1.8), compared to typical values reported for carnivorous species [74], suggests that our dietary Hg estimates may be overestimated due to these limitations.

Nevertheless, mean BAF and BMF > 1 implies that both prey and barn owl juveniles accumulate Hg compared to their environment (soil), and that Hg biomagnifies in the barn owl food chain [112,45]. A positive correlation (Spearman $\rho = 0.24$, $p < 0.001$) between feather and fur THg in the nests suggests that barn owls acquire Hg from their prey, supporting existing literature on the trophic transfer of Hg in avian food chains [1,108,112], including the terrestrial food web [61,85]. These results suggest that terrestrial avian top predators tend to have higher Hg intake and are exposed to MeHg because of their high trophic position in the food chain.

Despite these findings, we cannot exclude the potential direct

influence of environmental Hg. To further investigate this, we used spatial analysis to assess other potential Hg sources ensuring to encompass factors that could affect Hg accumulation in barn owls.

3.5. Spatial interpolation and exploration

The Swiss Pollutant Release and Transfer Registry (PRTR) database reported 22 point sources releasing between 0.008 and 52 kg of THg and five diffuse sources releasing between 4.3 and 269 kg of THg annually into air, water and wastewater in Switzerland in 2021. Fig. 4 maps only the point sources, as the coordinates for diffuse sources were unavailable. The interpolation map of moss THg (Fig. 4; using 2020 data modeled with EBK) indicates elevated moss THg values near Switzerland's borders, with the highest values along the southeastern border with Italy. The Hg deposition in Switzerland studied using moss is relatively low compared to neighboring France, that was reported to have the highest levels of THg in mosses in 2015 (0.032–0.63 mg/kg; $n = 441$), Germany (0.005–0.2 mg/kg; $n = 397$) and Italy (0.031–0.12 mg/kg; $n = 26$) [68]. To support further analysis, we extracted predicted moss THg values at the nest locations from the interpolation map. Cross-validation results, interpolation diagnostic and output maps of interpolation of moss THg are detailed in the SI (Table S7, Figure S1 and Figure S2).

Wetlands are considered “hotspots” for Hg methylation because wetland ecosystems with high proportion of organic soils and water-logged soils promote biomethylation, enhancing the bioavailability and biomagnification of Hg in food chains [111,26,54,65]. Our spatial analysis showed that 88 % of barn owl nests were located within 2 km of wetlands. Seven sites were located within a 5 km geodesic distance from PRTR-reported point sources in 2021.

The optimized hotspot analysis identifies statistically significant spatial clusters of high and low Hg values, whereas the optimized outlier analysis identifies values that are significantly different from those of their neighboring nests. For soil THg values (Figure S6), in the Northern Plateau we identified 12 sites as high-value clusters and 5 sites with significantly low soil THg values compared to the neighboring sites. Similarly, in the Central Plateau we found 5 sites as high-value clusters. In the Southern Plateau, we found 3 sites (in Valais, situated at southernmost part) as high-value clusters and one site as low outlier value among its neighboring sites. Elevated mean soil THg was observed in the high cluster of three sites in the Southern Plateau, located near a legacy Hg contamination site close to a heavily industrialized area in the city of Monthey. Notably, our dataset's highest soil THg value ($118 \pm 9.4 \mu\text{g kg}^{-1}$) was found in meadows near Monthey, approximately 5 km from several point sources, including former crude oil refinery, incineration plant, gypsum quarry, and a chlor-alkali plant (legacy Hg site) (Figure S7).

Soils near point sources such as mining sites and chlor-alkali plants have been reported to have elevated Hg [86,113]. The point sources (including historical emissions) have possibly contributed to the local atmospheric Hg and impacted soils, vegetation, and aquatic bodies within barn owl habitats. We identified clusters of high fur THg (averaged for each nest) in pellets collected in the Jura region ($n = 4$) and in the Central Plateau ($n = 2$) (Figure S8). Although no current or historical local Hg point sources were reported in the Jura region by the Swiss PRTR, a hazardous waste landfill used to be in this region [27,95]. This legacy site could still be a potential diffuse source impacting Hg levels in small mammals in the Jura region. The outlier and hotspot analyses showed clusters of low fur THg values in the Southern Plateau (high outlier $n = 1$). In the Northern Plateau, fur THg values were similar to other regions, except for one nest that was identified as a low outlier. Three nests, each in Central Plateau and Jura, were identified as low fur THg outliers. The outlier values observed within the clusters also support the outcome of our model B, that the prey number found in each pellet and prey composition could be significant sources of variation.

Feather THg values averaged for each nest (Figure S9) showed high-

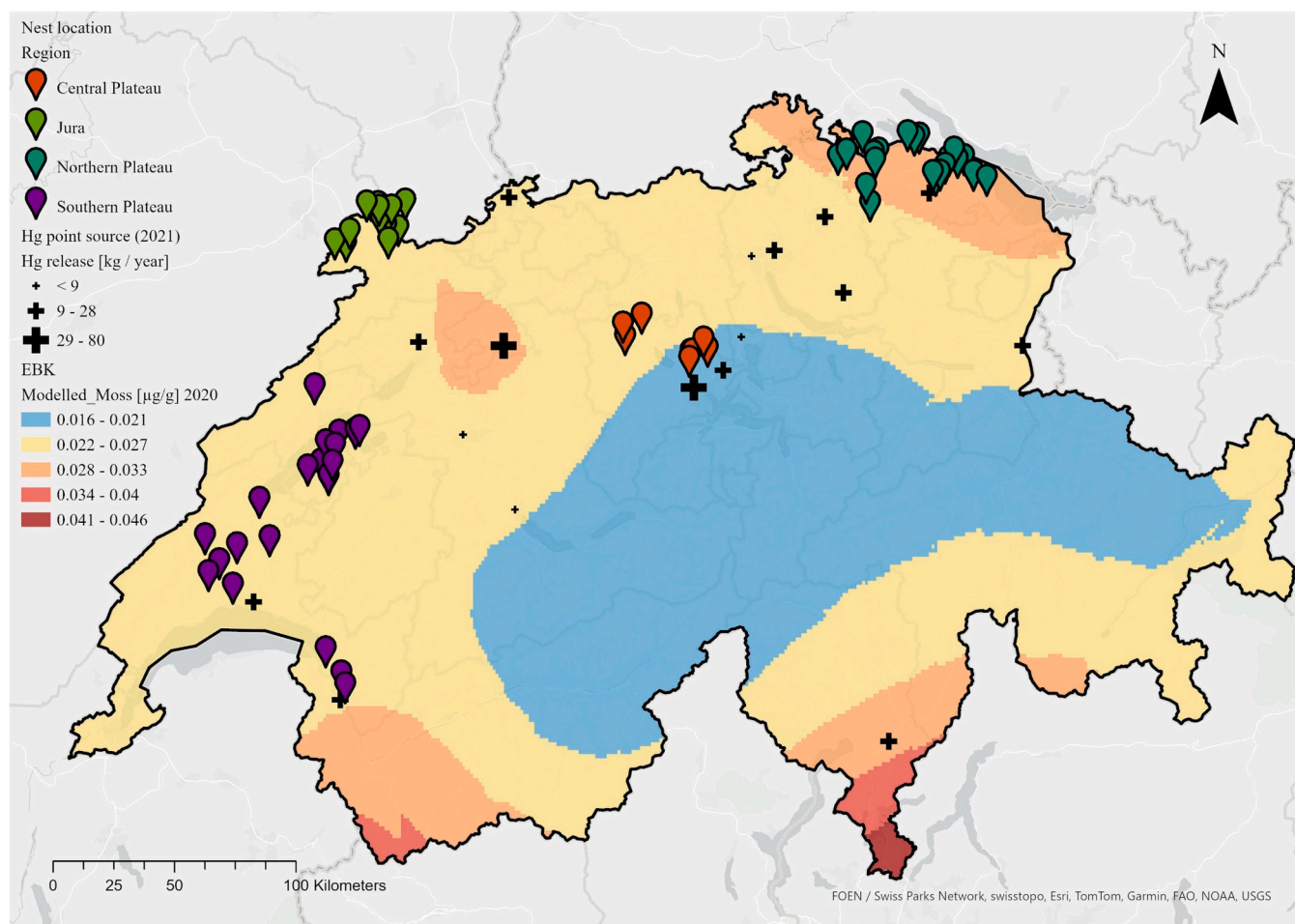


Fig. 4. Crosses represent Hg point sources, releasing varying amounts of THg (kg year^{-1}) in air, water and wastewater in 2021 (PRTR), with cross size proportional to release values. An interpolation map of moss THg ($\mu\text{g g}^{-1}$ dry weight) for 2020 was created using Empirical Bayesian kriging (EBK), based on measurements from the European Moss Survey (ICP Vegetation 2020) (details in [Section 2.4](#)).

value clusters in the Northern Plateau, suggesting barn owls in this region are exposed to elevated Hg levels. Two nests, one in the Jura and one in the Southern Plateau, were identified as high outliers within areas of generally low feather THg levels. Feather outlier analysis of individual feather THg revealed that some juvenile barn owls had significantly different feather THg than their siblings, supporting the individual's diet as a significant source of variation ([Table S9](#), Model C) [77,35]. However, it is also worth noting that overall health, differences in metabolism and feather growth rates could also influence individual feather THg, which were not accounted for in this study. Furthermore, nests with high feather THg corresponded to areas with higher moss THg ($0.029\text{--}0.037 \mu\text{g g}^{-1}$) ([Figure S10](#)). A positive significant correlation of soil THg with predicted moss THg at the nest site ([Table S8](#), Model A), combined with high soil THg clusters in the Northern Plateau ([Figure S6](#)) suggest that atmospheric deposition may be a primary source of Hg within the barn owl food chain. While waste recycling facilities act as local point sources, it is also plausible that transboundary atmospheric Hg from Germany contributes significantly to the observed Hg levels in the soil, moss and feathers in the Northern Plateau, given the documented pollution sources in the north-eastern border [99]. Additionally, Ballabio et al. [11] reported elevated soil THg in this area in Germany in the European Union topsoil Hg map. However, we could not identify any point release sources in the German PRTR in this area [96].

4. Conclusions

The global importance of this study supports Article 19 of the

Minamata convention on mercury [69] and helps to assess the effectiveness of such policies guiding international efforts to reduce exposure to wildlife and protect ecosystems. This study used a non-invasive sampling approach to quantify THg and MeHg in juvenile barn owls while investigating local contamination sources through their diet (fur from regurgitated pellets) and habitat. The low variation of THg in down feathers from juveniles of the same brood, together with consistent MeHg content, confirms that juvenile down feathers are reliable bio-indicators for Hg monitoring studies. Additionally, analyzing fur from regurgitated pellets provided valuable insight into Hg accumulation at lower trophic levels.

Spatial analysis complemented our study by highlighting local sources such as industrial and legacy contamination sites, and their association with elevated THg concentrations in moss (representing atmospheric Hg), soil and tissues in specific regions. The analysis also revealed potential transboundary Hg pollution impacting the local environments including the barn owl habitats. This analysis enabled us to further interpret our data and identify both local and regional sources of Hg pollution, as well as potential hotspots of Hg contamination that may require further investigation. Additionally, combining spatial assessments with statistical modeling (linear regression and mixed-effect models) enabled us to identify key environmental and biological variables that influence Hg accumulation and distribution.

This study offers a robust biomonitoring approach while contributing valuable data towards understanding Hg behavior in terrestrial ecosystems. Although current Hg levels in Switzerland are relatively low and Europe sees a reduction in Hg emissions, re-emission from legacy

contaminated sites, transboundary pollution, climate change and a predicted rise in global atmospheric Hg emissions driven by artisanal and small-scale gold mining activities [17] necessitate continued monitoring. Future studies should explore additional environmental and biological factors influencing Hg accumulation in the complex food webs of terrestrial top predators.

Environmental implication

This study highlights the significant role of dietary composition in Hg accumulation and biomagnification in the barn owl food chain. Avian apex predators, such as barn owl, serve as bioindicators of environmental Hg pollution and reflect contamination levels across multiple trophic levels. Species- and habitat-specific exposure in addition to the dietary preference influence Hg transfer. Our findings emphasize the need for continuous Hg monitoring in terrestrial ecosystems, particularly in areas near Hg pollution sources and wetlands. Expanding on this research is essential as it provides a framework for the early detection and long-term monitoring of Hg contamination in terrestrial ecosystems, while assessing the effectiveness of the Minamata Convention.

Research ethics

This work has received approval for research ethics from the Swiss Federal Food Safety and Veterinary Office (FSVO) (National authorization number: 33338) and Swiss Canton of Vaud's Directorate General for Agriculture, Viticulture and Veterinary Affairs (DGAV) (Cantonal authorization numbers: VD3658 and VD3658a). Proof of approval is available upon request.

CRedit authorship contribution statement

Mestrot Adrien: Writing – review & editing, Supervision, Resources, Funding acquisition, Data curation, Conceptualization. **Roulin Alexandre:** Writing – review & editing. **Buser Andreas M.:** Writing – review & editing. **Marmet Dan Samuel:** Writing – review & editing, Investigation, Formal analysis. **Heckel Gerald:** Writing – review & editing, Supervision, Resources, Methodology. **Almasi Bettina:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology. **Kjelsen Ingrid Sofia:** Writing – review & editing, Investigation, Formal analysis. **Mahat Sabnam:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Adrien Mestrot reports financial support was provided by Swiss Federal Office for the Environment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2025.138269](https://doi.org/10.1016/j.jhazmat.2025.138269).

Data availability

Data will be made available on request.

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