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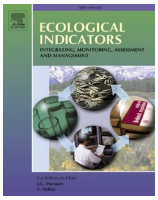

Small mammals as a bioindicator of mercury in a biodiversity hotspot – The Hengduan Mountains, China

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ABSTRACT

Due to toxicities, capacities for long-range transportation, bioaccumulation, and biomagnification, mercury (Hg) presents a unique concern to wildlife in remote ecosystems, including "the roof of the world". Large carnivorous predators are thought to be exposed to elevated Hg due to their high trophic positions, but the direct assessment for Hg contamination is a challenge. Given the poorly understood Hg exposure in these carnivores, establishing a reliable and straightforward assessment would be essential to identify targeted species at Hg exposure risk for effective conservation, particularly in fragile biodiversity hotspots. Small mammals are abundant and serve as prey for large top predators. Combined with local observations, we provided an assessment to estimate the daily Hg exposure via consumption of small mammals for large carnivorous mammals recorded in the Hengduan Mountains, a world biodiversity hotspot, China. Within an altitude span from 2043 to 4251 m a.s.l., the average topsoil total mercury concentration (hereafter [THg]) was 44.65 ± 25.80 μg/kg (mean ± sd; 10.54 – 135.15 μg/kg, n = 41), while the hair [THg] in small mammals was 104.66 ± 91.96 μg/kg (mean ± sd; 7.73 to 385.70 μg/kg, n = 13). Furthermore, the daily intake of Hg was calculated among the 22 investigated/historical-recorded carnivore mammals belonging to 5 families. We found a large variance in daily intake of Hg via small mammals: Felidae (median: 205.93 μg/day) > Ursidae (135.02 μg/day) > Canidae (92.92 μg/day) > Viverridae (19.88 μg/day) > Mustelidae (7.18 μg/day). Specifically, Tiger *Panthera tigris* was found with the highest Hg daily intake (1701.39 μg/day) via consuming small mammals, while species belonging to Mustelidae generally have low Hg daily intake (<3 μg/day). This study provides a feasible approach to identifying species at environmental Hg risk in this fragile remote high-elevation region. The limitations and future improvements were discussed.

1. Introduction

As a semi-volatile persistent pollutant, atmospheric mercury (Hg) can undergo long-distance transportation, and subsequently be redeposited across various ecosystems (Driscoll et al., 2013), including marine, aquatic and terrestrial environments (Douglas et al., 2012; Jackson, 1998; Tseng et al., 2021). Mercury poses adverse health impacts to organisms, partially due to its widespread methylation of Hg in anoxic environments, bioaccumulation, as well as biomagnification along food webs. Not just for humans, detrimental effects caused by Hg on immune response, reproduction, as well as fetal development have been identified in vertebrates (Chételat et al., 2020; Scheuhammer et al., 2007). Wildlife exposure to Hg particularly its organic form (methyl mercury, MeHg) have been widely documented, even in remote fragile regions (e.g., the Arctic, Dietz et al., 2022; Douglas et al., 2012). Mammals, with their diverse body sizes and foraging traits, play

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essential roles within ecosystems (Lacher et al., 2019), but they are also susceptible to the toxicity of Hg (Wren, 1986). Prior research has provided significant insights into the vulnerabilities and responses of mammalian species to environmental Hg exposure (Borchert et al., 2019; Dibbern et al., 2021; McGrew et al., 2014; Poddubnaya et al., 2021; Wren, 1986; but see the review of Dietz et al., 2022). To assess the efficacy of Hg emission control efforts and environmental release mitigation under the Minamata Convention on Mercury, it is crucial to focus Hg biomonitoring endeavors on terrestrial ecosystems of notable biodiversity significance (Wang et al., 2019a).

Mountain regions harbor approximately one-third of terrestrial biological diversity on Earth (Spehn et al., 2011). Meanwhile, serving as condensers for long-range atmospheric pollutants, high-altitude terrestrial mountains consistently receive wet and dry toxic depositions, via "mountain cold trapping effects" and "grasshopper effects" (Liu et al., 2014; Loewen et al., 2005; Wania and Mackay, 1995; Yu et al., 2022). Even within the "roof of the world" – the Tibetan Plateau and the Himalayas – Hg contamination persists within their intricate food webs (Liu et al., 2020; Xu et al., 2016). The terrestrial ecosystems in this region have been regarded as an important sink of Hg due to different geochemical and microbial characteristics (Liu et al., 2016). Additionally, recent studies found that Hg not just bioaccumulates in aquatic ecosystems (Zhang et al., 2014), but also in terrestrial ecosystems (Liu et al., 2020). The Hengduan Mountains, characterized by dramatic habitats and a diverse range of threatened species within a narrow vertical elevational gradient, have gained recognition as one of the 35 globally acknowledged biodiversity hotspots (Myers et al., 2000). Unfortunately, this region is situated in proximity to Hg emission sources carried by the Indian summer monsoon and westerly winds (Loewen et al., 2007, 2005; UN Environment, 2019). Even, certain insectivorous mammalian species such as the invertebrate-eating group *Soriculus*, have shown the ability to accumulate Hg to levels of concern (Ma et al., 2021), indicating the presence of Hg bioaccumulation and associated ecological risks in higher trophic level predators. It is imperative to promptly evaluate their exposure and ecological risks related to Hg from a conservation standpoint (Li et al., 2022).

It is widely acknowledged that the adverse effects caused by anthropogenic pollutants on wildlife could escalate to the extent of endangering sensitive species and subsequently diminishing biodiversity (Groh et al., 2022; Sigmund et al., 2022). Thus, for effective conservation strategies, it becomes crucial to pinpoint species of concern from an ecotoxicological perspective. However, quantifying pollutant levels in wildlife residing within a delicate biodiversity hotspot presents challenges, as these creatures are difficult to locate and sample-even for commonly used non-invasive materials like feces, hair, or feathers. Instead, an alternative approach involves indirect assessment through estimating daily intake from food consumption, a method well-established in human populations (Weihe et al., 1996) occasionally applied to select threatened flagship species (e.g., Giant Panda *Ailuropoda melanoleuca*), providing insights into ecological risk (Amusa et al., 2021; Chen et al., 2017, 2022; Zhao et al., 2020).

Due to their abundance, widespread occurrence, and local home range, small terrestrial mammals are key bioindicators for biomonitoring in environmental contamination (Ballová and Janiga, 2018; Ballová et al., 2020; Huckabee et al., 1973; Talmage and Walton, 1991; Turna Demir and Yavuz, 2020). Moreover, they are prey for large mammals and avian predators, playing a critical role in energy flow and material circulation within terrestrial food webs. To safeguard a biodiversity hotspot, the identification of targeted species that are exposed to environmental Hg through the consumption of edible small mammals could offer a potential avenue for gaining deeper insights into Hg contamination in threatened wildlife.

This study was undertaken with the goal of enhancing our comprehension of Hg contamination within a biodiversity hotspot in China. Our hypothesis centered on the idea that the exposure of predatory mammals to Hg could be gauged through the daily intake of small mammals. In particular, our objectives were as follows: 1) to determine the concentrations of Hg in surface soil and small mammalian hair located in the southern Hengduan Mountains of China; 2) to combine the investigation and dietary intake data from captive feeding experience to estimate the daily Hg exposure for the species documented within this region.

2. Materials and methods

2.1. Study site and sampling

During the summer of the year 2020, our study involved the collection of small mammals as part of a biodiversity investigation within the Hengduan Mountains. Our field works were conducted across three mountains named Boxiola Ling, Tenasserim Chain, and Mangkang Shan. These mountains are situated in the Southeast Tibet Plateau of China (as depicted in Fig. 1, spanning coordinates $26^\circ25' - 28^\circ27'$N, $91^\circ28' - 94^\circ22'$E), together with an elevational range between 2043 and 4251 m above sea level (m a.s.l.). Overall, within a warm and humid monsoon climate, there are five main vegetation zones across this elevational gradience: dry valley shrub at the low elevation band, broad-leaved deciduous forest; coniferous and broad-leaf mixed forest and dark coniferous forest at the middle elevation band; alpine shrub and meadow at high elevation band, respectively. Habitats were continuous along the elevational gradients and relatively undisturbed by human activities.

2.2. Terrestrial carnivorous mammal investigation

A total of 38 camera traps and 37 line intersects were conducted focusing on medium and large-bodied mammals and 24 camera traps were retrieved after 14 months from Sep 2019 to Oct 2020. The identification of taxonomy of mammals followed by Jiang et al. (2015). In terms of literature on carnivores recorded in this region, we searched literature focused on the Tibet Plateau and the Himalayas (Feng et al., 1980a, Feng et al., 1980b; Huang et al., 2008; Jiang et al., 2015).

2.3. Analytical methods

The sampling procedure for both topsoil and small mammals was consistent with the methodology detailed in Ma et al. (2021). Soil samples were freeze-dried and manually ground, then sieved through a 75 μm mesh to remove large debris before further analysis. Small mammals were extracted from the traps, and their biological metrics were measured. Hair samples were taken from the upper back using scissors, carefully placed in paper envelopes, and stored at ambient temperature before being transported to the laboratory. Prior to analysis, hair samples were cleaned by washing with acetone, rinsing with D. I. water, and air drying.

The analysis of soil and hair samples for total Hg concentration (hereafter [THg]) was performed via a Direct Mercury Analyzer (DMA 80, Milestone Inc., Italy), following the US Environmental Protection Agency Method 7473 (EPA, 1998) at the State Key Laboratory of Environmental Geochemistry, China. The analytical quality was controlled by system and method blanks, the certified materials (GSS-5, soil, National Research Center for Standards in China and DORM-4, fish protein, National Research Council Canada), and a duplicate sample with each batch of 10 or fewer samples. For analysis, at least 10 mg of hair was directly placed into a weighing boat to determine Hg concentrations. The method detection limit for the DMA 80 is 0.0008 μg/g dry weight. Relative percentage differences (mean ± sd) for duplicate samples were 2.04 ± 1.27% ($n = 4$). Recoveries of GSS-5 was 95 ± 0.01% ($n = 4$), while DORM-4 was 100 ± 0.01% ($n = 4$). All Hg analyses met the measurement quality objectives under the certified methods.

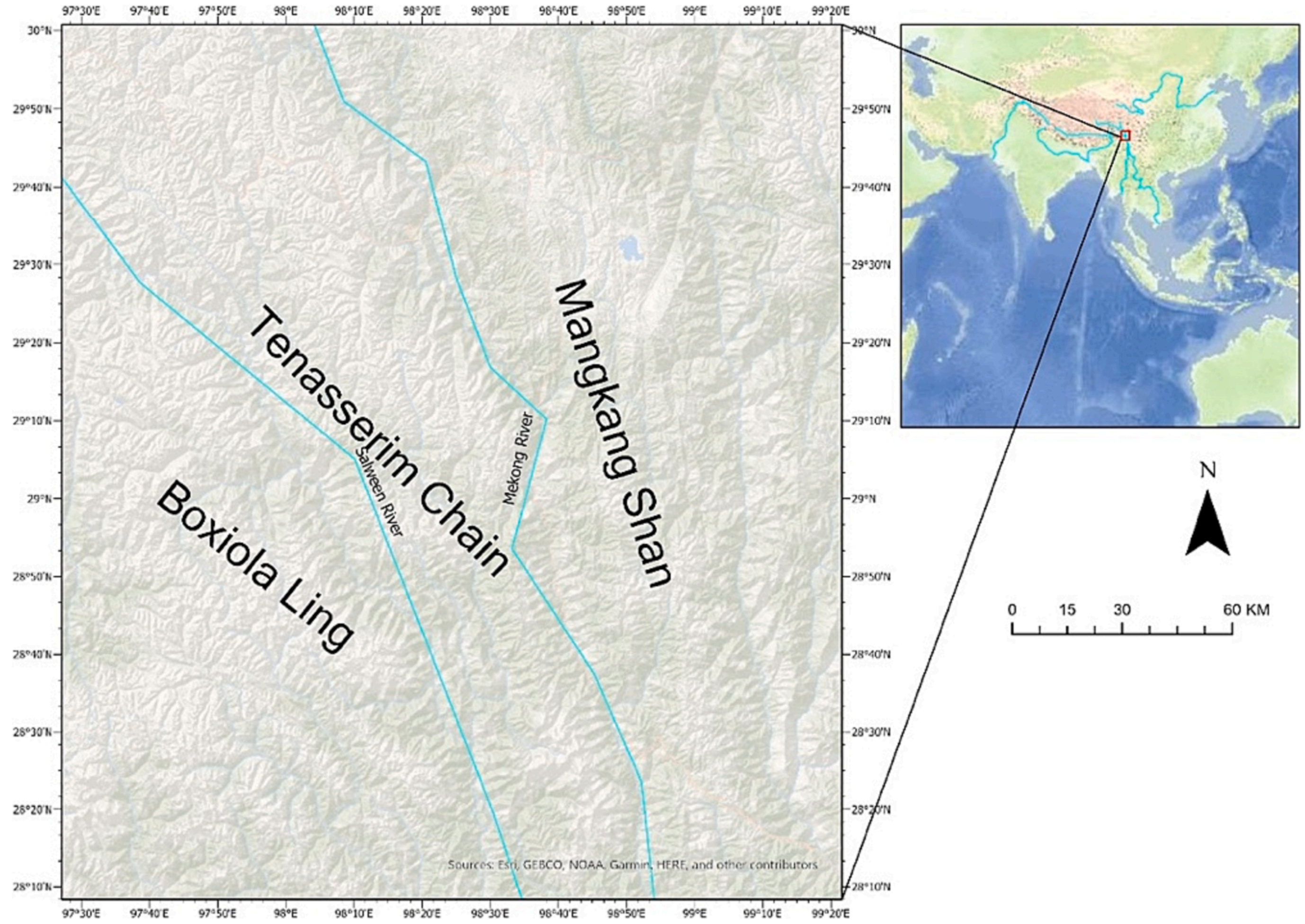

Fig. 1. The study site of the Hengduan Mountains, the Southeast Tibet Plateau of China.

2.4. *Dietary Hg exposure estimates for top predators*

Combined with our field and literature investigation, we documented large mammalian species within our designated region. In particular, quantifying the dietary intake of Hg by large species through the consumption of small mammals involves a multi-step calculation. This process begins by multiplying the conventional average concentration of Hg within their bodies (for which we employed the average hair Hg concentration) by the minimum weight of the consumed species, and their diet percentage according to attributes described in Wilman et al. (2014). This weight was conservatively set at 10% of the predator's body weight (personal communication from Guangzhou Zoo, China).

2.5. *Statistical analysis*

We reported [THg] as arithmetic mean ± standard deviation (μg/kg). Polynomial regression analyses were performed to assess the form of the patterns of soil [THg] as a function of elevation along the gradient. Order 1 equation indicates a linear pattern, whereas order 2 and order 3 equations indicate a unimodal pattern (hump-shaped pattern). All statistical analyses and visualization were conducted via R version 4.0.2 (R Core Team, 2020).

3. Results

3.1. *[THg]s in soil and small mammals*

Mercury content was detected in both the soil and hair of all small mammals collected in the southern Hengduan Mountains. The average of soil [THg] in Tibet E.S. is 44.65 ± 25.0 μg/kg (range: 10.54 ~ 135.15 μg/kg, n = 41). The geographical distribution of soil [THg] was shown in Fig. 2. In particular, soil [THg] in this research appears to be comparable to those documented in the Tibetan and Himalayan high altitudes (Ma et al., 2021; Tripathee et al., 2019). The polynomial (linear, quadratic, and cubic) regressions suggested no obvious patterns of soil [THg] along elevational gradience in our study region ($P > 0.05$).

Due to the limitations of being conducted as a side project, our data collection efforts resulted in the capture of only 13 individuals, predominantly herbivorous species. The descriptive statistics of [THg]s according to their species, sex, and sampling site are summarized in Table 1. On average, the [THg]s in the hair of the small mammals was recorded at 104.66 ± 91.96 μg/kg, encompassing a range of 7.73 to 385.70 μg/kg (n = 13, fresh weight). Specifically, the obligate herbivore Plateau Pika *Ochotona curzonia* collected in 3871 m a.s.l. had the lowest hair [THg]. Conversely, the Large-eared Field Mouse *Apodemus latronum* caught at 3579 m a.s.l. had the highest hair [THg]. The Large-eared Field Mouse, a member of the Muridae family, predominantly resides in regions spanning southwestern China, as well as southeastern Tibet and northern Burma (Jiang et al., 2015). This species exhibits a dietary preference for plant matter such as grass, grass seeds, and leaves, while also supplementing its diet with occasional insect consumption.

3.2. *Estimation of Hg daily intake by top predators*

Through a comprehensive review of literature and fieldwork, we identified a total of 23 carnivorous species across 6 families inhabiting

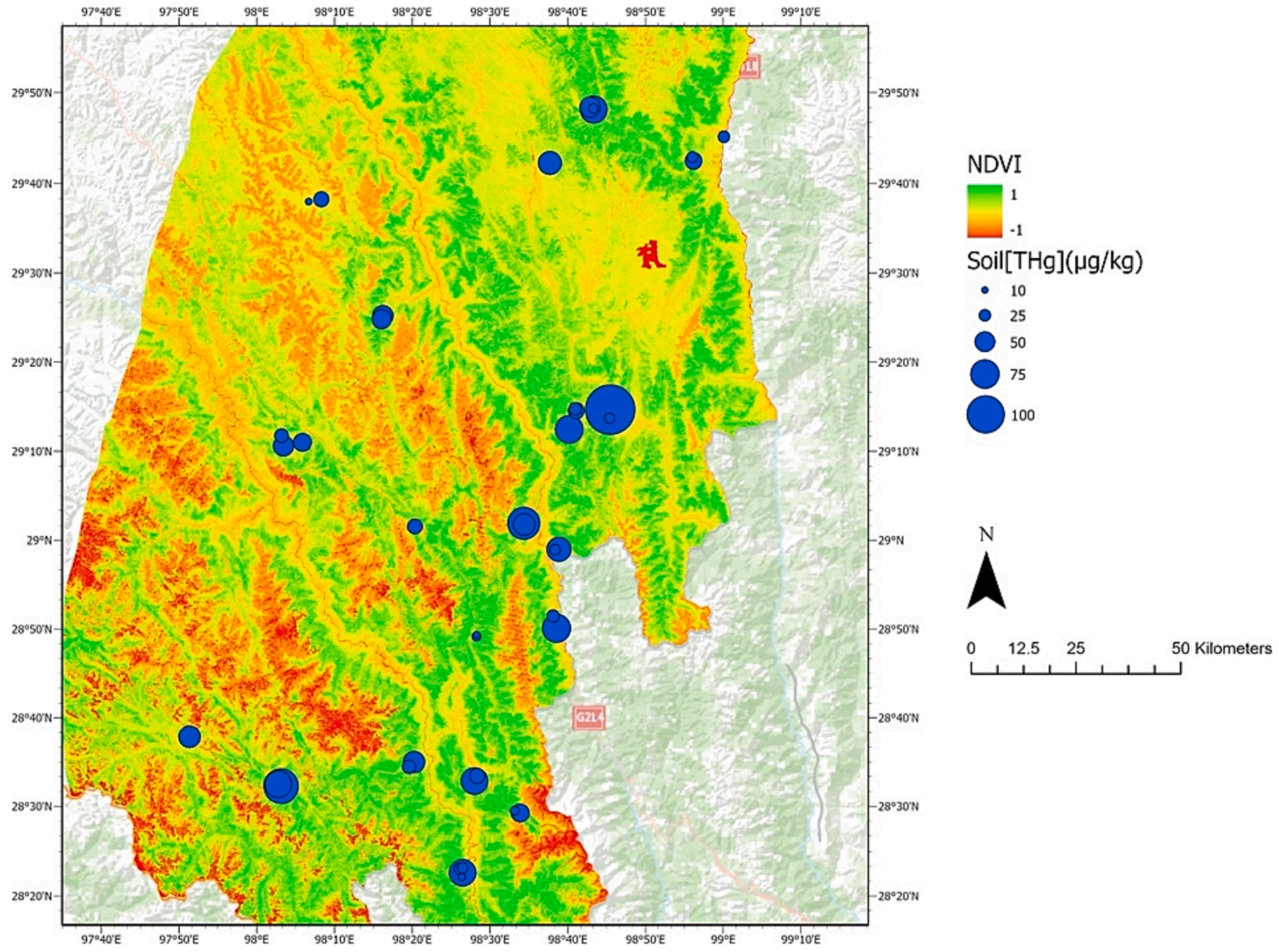

Fig. 2. Topsoil [THg]s sampled in the Hengduan Mountains, China.

Table 1
The descriptive statistics of [THg]s in captured small mammals according to their species, sex, and sampling location. HB (unit: mm): Length of head and body; T (unit: mm): Length of tail; HF (unit: mm): Length of hind foot; E (unit: mm): Length of ear; GLS (unit: mm): Greatest length of skull; BW (unit: gram): Body weight.

Altitude (m.a.s.l.)	Common name	Scientific name	HB	T	HF	GLS	E	BW	Hair [THg] (μg /kg)
3917	Large-eared Field Mouse	*Apodemus latronum*	90.8	100.2	23.6	33.6	19.8	27.1	88.01
3917	Large-eared Field Mouse	*Apodemus latronum*	90.6	96.1	23	30.7	18.0	23.4	61.30
3479	Chinese White-bellied Rat	*Niviventer confucianus*	138.8	177.1	30.2	24.7	42.8	75.7	75.92
3479	Large-eared Field Mouse	*Apodemus latronum*	111.2	85.0	22.2	20.3	34.5	39.4	84.47
3579	Large-eared Field Mouse	*Apodemus latronum*	82.7	96.7	23.6	20.4	32.0	23.2	90.87
3479	Large-eared Field Mouse	*Apodemus latronum*	103.8	95.0	25.3	20.9	30.4	23.8	83.13
3579	Large-eared Field Mouse	*Apodemus latronum*	100.3	110.8	25.2	21.3	33.6	32.1	385.70
2330	Chinese White-bellied Rat	*Niviventer confucianus*	112.1	149.4	26.1	22.3	39.5	47.4	162.88
2535	Grey Red-backed Vole	*Myodes rufocanus*	89.9	60.5	22.1	11.9	23.0	20.6	52.85
4247	Plateau Pika	*Ochotona curzoniae*	133.7	0	32.8	22.9	46.2	181.2	7.73
2330	Chinese White-bellied Rat	*Niviventer confucianus*	146.5	171.1	26.0	23.1	38.3	74.0	98.19
3917	Large-eared Field Mouse	*Apodemus latronum*	83.8	82.1	23.8	22.1	30.5	22.0	51.98
3917	South China Field Mouse	*Apodemus draco*	84.6	91.0	23.2	21.5	31.9	24.4	117.59

this biodiverse hotspot (Table 2). According to the IUCN Red List of Threaten Species (https://www.iucnredlist.org, access by 2023–01-13), Tiger *Panthera tigris* and Jackal *Cuon alpinus* are categorized as Endangered (EN) in the IUCN Red List of Threatened Species. Others fall under the categories of Near Threaten (NT, n = 2), Vulnerable (VU, n = 6) or Least Concern (LC, n = 12). Notably, even within the LC category, three species display declining population trends. Using available dietary percentage data for mammals from Wilman et al. (2014), we calculated the daily intake of [THg]. The estimated Hg exposure among recorded carnivore species exhibited a range of variation. Felidae displayed the highest risk of Hg intake (median: 205.93 μg/day), followed by Ursidae (median: 135.02 μg/day), Canidae (median: 92.92 μg/day), Viverridae (median: 19.88 μg/day), then Mustelidae (median: 7.18 μg/day). Notably, even within the Felidae family, the Tiger, the apex predators of ecological pyramids, exhibited the highest daily mercury intake at 1701.39 μg/day, a value approximately 70 times greater than that of the

Table 2
Data of diet and body weight for the investigated carnivore mammals at the Hengduan Mountains in this study (Wilman et al., 2014). L: literature; F: field investigation. CR: Critically Rared; EN: Endangered; LC: Least Concern; NT: Near Threatened; VU: Vulnerable; BW: body weight. ↓: Population Decreasing; -: Population Stable;?: Population Unknown; *: data from Wilson and Mittermeier, (2009). F: fieldwork; L: literature review.

Species	Scientific name	Family	Data source	IUCN category	% of mammalian diet	BW (kg)	minimum daily intake (kg)	Estimated Hg daily intake via small mammals (μg/day)
Asiatic Golden Cat	*Catopuma temminckii*	Felidae	L	NT (↓)	80	11.50	1.15	96.29
Leopard Cat	*Prionailurus bengalensis*	Felidae	F	LC (-)	70	3.30	0.33	24.18
Clouded Leopard	*Neofelis nebulosa*	Felidae	L	VU (↓)	100	19.68	1.97	205.93
Leopard	*Panthera pardus*	Felidae	L	VU (↓)	100	52.04	5.20	544.63
Tiger	*Panthera tigris*	Felidae	L	EN (↓)	100	162.56	16.26	1701.39
Snow Leopard	*Panthera uncia*	Felidae	L	VU (↓)	100	37.00	3.70	387.24
Masked Civet	*Paguma larvata*	Viverridae	F	LC (↓)	60	4.30	0.43	27.00
Large Indian Civet	*Viverra zibetha*	Viverridae	L	LC (↓)	20	9.50	0.95	19.89
Small Indian Civet	*Viverricula indica*	Viverridae	L	LC (-)	40	2.91	0.29	12.17
Wolf	*Canis lupus*	Canidae	F	LC (-)	100	32.18	3.22	336.83
Jackal	*Cuon alpinus*	Canidae	L	EN (↓)	90	14.17	1.42	133.50
Tibetan Fox	*Vulpes ferrilata*	Canidae	F	LC (?)	100	5.00	0.50	52.33
Red Fox	*Vulpes vulpes*	Canidae	F	LC (-)	70	5.48	0.55	40.12
Brown Bear	*Ursus arctos*	Ursidae	L	LC (-)	10	180.52	18.05	188.93
Asian Black Bear	*Ursus thibetanus*	Ursidae	F	VU (↓)	10	77.50	7.75	81.11
Chinese Red Panda*	*Ailurus stayni*	Ailuridae	L	NA	NA	4.50	0.45	NA
Small-Clawed Otter	*Aonyx cinerea*	Mustelidae	L	VU (↓)	0	3.53	0.35	0.00
Greater Hog Badger	*Arctonyx collaris*	Mustelidae	L	VU (↓)	10	6.36	0.64	6.65
Asian Badger	*Meles leucurus*	Mustelidae	L	LC (?)	30	13.00	1.30	40.82
Yellow-Throated Marten	*Martes flavigula*	Mustelidae	F	LC (↓)	40	1.84	0.18	7.72
Beech Marten	*Martes foina*	Mustelidae	L	LC (-)	50	1.54	0.15	8.06
Alpine Weasel	*Mustela altaica*	Mustelidae	F	NT (↓)	100	0.17	0.02	1.79
Yellow Weasel	*Mustela sibirica*	Mustelidae	F	LC (-)	70	0.41	0.04	2.97

Leopard Cat *Prionailurus bengalensis* with an intake of 24.18 μg/day. Among the carnivores, the weasel group composed of the Alpine Weasel *Mustela altaica* and Yellow Weasel *Mustela sibirica* exhibited the lowest Hg intake, amounting to less than 3 μg/day. Additionally, it's noteworthy that the Ailuridae (Chinese Red Panda *Ailurus stayni*) and the Small-Clawed Otter *Aonyx cinerea*, lacking diet proportions of terrestrial small mammals, might still face Hg exposure through other prey sources such as invertebrates and fish (Jiang et al., 2015).

4. Discussion

4.1. *Geographical distribution of soil [THg]s*

Our collected data serves as a fundamental reference for understanding Hg contamination trends across the broad spectrum of elevations within a significant biodiversity hotspot. In contrast to the distinctive hump-shaped pattern witnessed in the mountain-valley system of the East Himalayas (Ma et al., 2021), the distribution of soil [THg] in our study area did not manifest an elevation-dependent trend. This inconsistency has also been observed in other metrics through prior studies. For instance, a pattern emerged where Hg concentrations in snow increased with altitude in the southern Tibetan Plateau (Huang et al., 2012). However, in both the central (Paudyal et al., 2017) and northern (Huang et al., 2014) regions of the Tibetan Plateau, no clear correlations were found. This complexity stems from various factors, including geographical location, atmospheric deposition, the composition of vegetation (Yu et al., 2022); vegetation foliage (Ballabio et al., 2021); soil organic matter (Ma et al., 2021); and some anthropogenic impacts such as climate change (Wang et al., 2019b). This pursuit of understanding promises insights into the complex matrix of variables influencing [THg] variability within the third pole region, shedding light on its unique ecological intricacies.

4.2. *Small mammalian [THg]s among high altitudes*

Several studies examined [THg]s in small mammals populations across different regions, including investigations conducted in the High Tatra Mountains in Central Europe (Ballová et al., 2020; Martinková et al., 2019), the Lebu Valley, East Himalayas of China (Ma et al., 2021). Remarkably, our collected data aligns closely with the [THg] range observed in rodents (ranging from 8 μg/kg to 300 μg/kg) from areas affected by Hg-containing coal combustion in Wyoming, USA (Huckabee et al., 1973). Moreover, our results either correspond to or even exceed the hair Hg concentrations found in primary herbivorous small mammals (8.3 μg/kg to 183.0 μg/kg) inhabiting the Lebu Valley, as well as desert-dwelling species (ranging from 6.8 μg/kg to 164.7 μg/kg) collected near the Las Vegas valley (Gerstenberger et al., 2006). These findings highlight the global nature of Hg contamination and its prevalence across various ecosystems.

4.3. *Methodological limitations*

It is essential to acknowledge the limitations inherent in our approach. Firstly, a significant challenge arises from the absence of a direct correlation between body burden and hair concentrations in small mammals, which impedes the development of equations to accurately calculate edible tissue concentrations. Secondly, the daily intake of pollutants in free-living wildlife may significantly differ from that of captive counterparts. As a result, future investigations could explore potential corrections between these two groups to refine our estimations. Thirdly, the establishment of regulations for ecological safety based on daily intake in wildlife remains a crucial gap in our understanding. Such regulations would be instrumental in formulating effective conservation strategies to safeguard wildlife populations from Hg contamination. Lastly, exposure to Hg predominantly occurs via the dietary pathways for mammals (Eisler, 1987; Wiener, 2013). Nonetheless, it is important to recognize that other intake pathways, namely inhalation and dermal contact (Clarkson and Magos, 2006) were not considered here. Additionally, according to recent studies (Tsz-Ki Tsui et al., 2019; Yung et al., 2019), elevated MeHg content could be generated during litterfall decomposition processes and dermal contact could be a pathway for small mammals burrow. Thus, apex predators which consuming small mammals, could be exposed to elevated MeHg along lengthen food chains (Manlick et al., 2023). These considerations highlight the multifaceted nature of Hg exposure pathways and underscore the importance of further research to fully grasp the ecological implications.

5. Ecological implication and conclusion

Our study demonstrates that small mammals can be used to estimate the daily intake of Hg in their predatory species combining with local records and aim to identify target species that needs more attention to pollution threat in fragile ecosystems. From a wildlife health perspective, trophic transfer of Hg to advanced vertebrate carnivorous mammals is particularly of interest since large carnivorous mammalian species are likely threatened species. The accumulated in human health associated with Hg pollution was estimated as $19 trillion during 2010–2050 (Zhang et al., 2021). Yet, the biodiversity loss regarding to wildlife particularly threaten species due to Hg toxicity has not been well estimated (Groh et al., 2022). Combining the investigation record and dietary intake data from captive feeding experience, we estimated the daily Hg exposure for the predatory species recorded in this region. Our approach provides a quick and straightforward assessment to identify targeted carnivorous species potentially at Hg exposure risk. This baseline information would be useful to further research plan and conservation strategy.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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