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BONES OF FREE-LIVING UNGULATES AS INDICATORS OF ENVIRONMENTAL CONTAMINATION BY MERCURY: A COMPARATIVE STUDY BETWEEN THE WESTERN CARPATHIANS AND ZHONGAR ALATAU

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Abstract

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We monitored the variability of mercury concentration in bones from naturally deceased or predator-caught ungulates from the Western Carpathians (Slovakia) and Zhongar Alatau (Kazakhstan), including red deer, roe deer, Tatra chamois, horse, Asiatic ibex, Marco Polo sheep, and other Bovidae. These species, as herbivores, have the advantage of a low trophic level, which implies a lower degree of mercury accumulation in the food chain. We compared the extent to which concentrations differed by location and habitat type. Mercury concentrations were determined by a DMA-80 analyzer. Lower mean concentrations observed in bones from Kazakhstan (0.0024 mg kg^{-1} dry weight) than from Slovakia (0.0063 mg kg^{-1} dry weight) suggest a lower level of ecosystem contamination from anthropogenic sources. The influence of habitat proved to be nonsignificant. Although bones are not a target organ for mercury accumulation, they reflect the state of environmental pollution and can be used in noninvasive retrospective environmental monitoring methods.

Key words: Tatra chamois, red deer, roe deer, alpine habitat, forest habitat, herbivores.

Introduction

Mercury (Hg) is a heavy metal, a pollutant that is toxic at already relatively low concentrations. It occurs naturally in bedrock, from where it is released during volcanic activity (Jitaru, Adams, 2004). It is estimated that annually up to 5,000 tonnes of mercury are released into the atmosphere, and an estimated 2,320 tonnes are of anthropogenic origin from sources such as fossil fuel combustion, waste incineration, nonferrous metallurgy, gold mining, cement production, and caustic soda production (Pirrone et al., 2010). Atmospheric mercury can be transported over long distances, and it also can overcome chemical changes. Elemental mercury in the atmosphere oxidizes to the form of mercurous (Hg^{+}) (or more often mercuric (Hg^{2+})) cation and combines with salt anions (chlorides, oxides, sulfides). Divalent cation is more soluble in water. Less reactive and insoluble particles are eliminated from the atmosphere by the settling gravity effect and adhesion to the surface of objects, water bodies, soil, and vegetation (Jitaru, Adams, 2004). In water, soil, and sediments, deposited Hg^{2+} from the atmosphere can undergo chemical reactions. It can be reduced to Hg^{0} or methylated to methylmercury (MeHg) with the participation of sulfate-reducing and iron-reducing bacteria (Gerson et al., 2017).

High-elevation ecosystems are particularly vulnerable (Ballová, Janiga, 2018) and susceptible to pollution by atmospheric contaminants due to orography, meteorological conditions, and low capabilities for self-purification and self-recovery (Zhang et al., 2013). The orography of mountain ranges and high altitudes act as a barrier to atmospheric contaminant transport (Townsend et al., 2014; Gerson et al., 2017; Bing et al., 2018; Tripathee et al., 2020; Ma et al., 2021). Higher elevations, especially their windward side, deposit contaminants from long-range transport (Bing et al., 2018). Higher rainfall frequency at high altitudes contributes to higher rates of removal of reactive species from the atmosphere by wet deposition (Tripathee et al., 2020). The humid and aqueous environment is more conducive to the activity of methylating bacteria (Gochfeld, 2003). However, the methylation mechanism also operates outside of a permanent water source (Townsend et al., 2014) in seasonal ponds and moist soils (Gerson et al., 2017). Low temperatures reduce evaporation (Zhang et al., 2013), which also contributes to higher soil Hg concentrations at higher elevations. In contrast, arid regions with deficient rainfall exhibit lower pollution rates (Yang et al., 2023). In arid regions, deposition is mainly influenced by atmospheric concentrations, not precipitation (Tripathee et al., 2020).

Large herbivores such as ungulates are often used as bioindicators in ecotoxicological monitoring. The advantages of using ungulates such as roe deer and red deer are their abundance in temperate climates (Giżejewska et al., 2016, 2017; Cappelli et al., 2020), well-known biology, and feeding habits (Giżejewska et al., 2017). However, they inhabit different habitats and can change ecological habits (Demesko et al., 2019). For example, these species may live in the vicinity of human settlements (Giżejewska et al., 2017), have a relatively small home range, are sedentary, and do not migrate for long distances (Giżejewska et al., 2017; Chyla et al., 1996). As herbivores, they better reflect environmental contamination as the impact of bioaccumulation in the food chain is limited by their lower trophic levels, especially in the case of metals such as mercury, with the property to accumulate at higher concentrations in higher order consumers (e.g., Gnamuš et al., 2000; Kalisinska et al., 2021; Komov et al., 2017).

We decided to compare differences in mercury concentrations at two different sites—the Western Carpathians (Slovakia) and Zhongar Alatau National Park (Kazakhstan). The area in Central Asia is located far from the ocean, and the surrounding mountains prevent moisture access, creating one of the largest arid regions in the world (Yang et al., 2023). Kazakhstan was ranked as the eighth largest emitter of Hg in 2000 (Pacyna et al., 2006), with emissions of up to 43.9 tonnes per year. In 2010, UNEP estimated Kazakhstan's emissions at 18.7 tonnes per year (Karaca et al., 2021). Cases of high pollution by mercury were reported in the Nura River, caused by the acetaldehyde plant, and in Pavlodar, originating from the chlor-alkali plant (Karaca et al., 2021). Up to 1.5 kg of metallic Hg is present in 1 tonne of sodium hydroxide produced (Guney et al., 2021). The distribution of emissions extends to Mongolia, northwest China, and southwest Kazakhstan (Guney et al., 2021). In addition to their own sources, countries are affected by long-range transport from East Asia, where up to 15% of particulate Hg may come from this source, and in case of reactive Hg, it is even more (Chen et al., 2014). In addition to active operational pollution sources, there is a persistent burden from closed plants. Contaminants from the mining, smelting, and military industries, active during World War II and the USSR era, remained in the soil after the closure of the operations (Yang et al., 2023). In Central Europe, the largest anthropogenic sources of pollution are nonferrous metallurgy in Bohemia, a thermometer factory in Warsaw, and the remains of mining activity in Rudňany (Gworek et al., 2020). In Slovakia, the average concentration of Hg in soil is 0.19 mg kg^{-1}, which is the second highest average in Europe (Panagos et al., 2021). The high level of mercury contamination of mountain ecosystems in Central Europe has been demonstrated by several studies (e.g., Maňkovská et al., 2008; Kompišová Ballová et al., 2020). Emissions in Slovakia originate from domestic sources (47%), natural sources (13%), and the remaining emissions are of transboundary origin, with most (16%) coming from Poland (Ryaboshapko et al., 1998).

In our research, we investigated the Hg abundance in the bones of ungulates. Even though bone appears to be an inert tissue, it is capable of regenerating (Florencio-Silva et al., 2015), in which trace elements from the bones are translocated to other organs or released into the blood and subsequently excreted from the body. The rate of bone regeneration is slower than that of soft tissue, and it is estimated that about 10% of bone tissue is regenerated annually in humans (Demesko et al., 2018). This slow turnover rate makes bone tissue more persistent than soft tissue; it is therefore a suitable material for tracking and monitoring long-term exposure to metals (Zaccaroni et al., 2008; Lanocha et al., 2012, 2013; Demesko et al., 2018). We chose bone material because of its availability in the field. Collection of fresh material from live specimens of species such as Tatra chamois, Asiatic ibex, and Marco Polo sheep would have been difficult due to the protected status of these species. We used material from an already existing collection of bones of Slovak ungulates—deer, roe deer, and Tatra chamois. Red deer and roe deer have similar food preferences. They feed on herbs, woody plants, wild fruits, or occasionally on agricultural crops. Tatra chamois feeds on herbs and lichens, growing in a higher altitude zone. For comparison, in Zhongar Alatau National Park, we collected bones of species that are the ecological equivalent of these species—deer, horses, Asiatic ibex, and Marco Polo sheep. All species as herbivores belong to the same food guild by feeding preferences, and therefore, we assume similar physiological characteristics, determining the degree of mercury contamination (Gnamuš et al., 2000). Thus, detected differences in bone contamination mainly reflect differences in environmental contamination. We can also detect whether differences in habitat where species live also affect mercury accumulation in bones.

Material and methods

Site characteristics and sample collection

Collected bones originate from two localities—Slovakia and southeast Kazakhstan. Bones from Kazakhstan were collected around Zhongar Alatau National Park (N45.035946, E79.963489). This area of 356,002 ha was declared as a national park in 2010. According to climatic properties, the area is characterized as cold and semi-arid. Higher altitudes receive more precipitation and are more humid and colder. Habitats where bones were found include treeline ecotone, alpine meadow, and rocky scree habitats. The altitude range is 1554–3217 m a.s.l. Bones were identified as the remains of 8 horses (*Equus caballus*), 3 deer (family Cervidae), 1 Asiatic ibex (*Capra sibirica*), 1 Marco Polo sheep (*Ovis ammon polii*), and 7 unidentified Bovidae (sheep, goats or ibex).

Bones of Slovak ruminants—red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and Tatra chamois (*Rupicapra rupicapra tatrica*)—originate from collections stored at the Institute of High Mountain Biology. Samples of red deer and roe deer bones were found in mountain regions of the Western Carpathians in northern Slovakia in the vegetation zone of deciduous and montane coniferous forest. Ten roe deer bones originate from Great Fatra mountains near the town of Ružomberok (49.067667, 19.260052), 11 red deer bones were found in Belianske Tatry in an eastern part of the High Tatras (49.254178, 20.207478), and one red deer sample was found near Demänovská valley in the Low Tatras (49.002599, 19.581103). The collection site of two red deer samples was unspecified. Most of the 58 bone remains of chamois originate from the Tatra population, inhabiting an alpine habitat, but there were also three bone samples of chamois

Table 1. Descriptive statistics of T-Hg (mg kg^{-1} dry weight) in bones.

		Mean	SD	Min	Max	n	test	p
Locality	Slovakia	0.0063	0.0089	0.0006	0.0560	82	Mann-Whitney	0.0062
	Kazakhstan	0.0024	0.0019	0.0003	0.0070	20		
Habitat (SK)	Alpine	0.0055	0.0082	0.0006	0.0560	58	Mann-Whitney	0.4206
	Forest	0.0083	0.0103	0.0006	0.0358	24		
Bone type (SK)	Limb	0.0077	0.0101	0.0006	0.0056	59	Mann-Whitney	0.0056
	Axis skeleton	0.0029	0.0023	0.0006	0.0100	23		

from a geographically isolated population in the Low Tatras that shared the same alpine habitat. The population in the Low Tatras may include hybrids with the subspecies *R. rupicapra tatrica.*

Laboratory analysis

Bones were mechanically cleaned by brush to remove possible surface impurities. A piece of bone tissue from the surface of the bone was separated mechanically with pincers and manually crushed using mortar and pestle into adequately small pieces. A sample of not more than 0.01 g was thus obtained. Precise weight was determined using the balance KERN 770 (KERN, Germany). The crushing of each sample into small particles was important because of the technology used to determine the mercury concentration. The DMA-80 technology (Milestone, Italy) uses the principle of spectrometry combined with the properties of mercury to form amalgams with gold. The first stage of the analytical process involves the combustion of the sample at 650 °C. To burn all the sample, the fineness of the material is important. Subsequently, all the mercury contained in the vapors formed reacts with the gold to form amalgams. Depending on how much amalgam is formed, we can detect the exact mass of mercury present in the sample. Temperature of the catalyst was set to 615 °C and cuvette temperature to 125 °C. The resulting concentration value (mg kg^{-1}) is obtained by calculating the mass of total mercury (T-Hg) in the sample and the mass of the sample. This technology determines the amount of all mercury in the sample, but it does not distinguish between inorganic mercury and its methylated form. The accuracy of the measurement was verified using a certified standard reference material of Beef liver NCS ZC 7001 (CHNACIS, China). An empty run was processed for every fifth measurement. This empty run allowed us to clean the apparatus and remove residual amounts of mercury, which can otherwise affect the results in the following sample.

Statistical analysis

At first, bones were divided into two groups—those collected in Slovakia and in Kazakhstan. Bones from Slovakia were also divided by habitat (alpine/forest) and by bone type (limb/axis skeleton). Combining all Slovak samples, collected in different habitats, and comparing them with the Kazakh group, which was strongly dominated by treeless alpine, could potentially bias the results. To eliminate this potential source of variability, an additional comparison was made between Kazakh groups and Slovak animals from alpine zone. Normality of groups was tested using the Shapiro–Wilk test. As none of the groups had a normal distribution, the nonparametric Mann—Whitney test was used to compare two groups. All statistical analyses were performed using PAST 4.16 (Hammer et al., 2001). The significance threshold was set at 0.05.

Results

Descriptive statistics of groups are summarized in Table 1. Concentrations of T-Hg (mg kg^{-1} dry weight) of bones collected in Slovakia differed significantly from bones collected in Kazakhstan ($p=0.0062$) (Mann–Whitney U test). Bones from Zhongar Alatau also differed from group of Slovak alpine bones ($p=0.0110$) (Mann–Whitney U test) and group of Slovak forest group ($p=0.0161$) (Mann–Whitney U test). The effect of habitat on mercury accumulation was tested in bones found in Slovakia. Red deer and roe deer are species from a forest habitat, whereas chamois inhabit the high-altitude alpine zone. Mercury levels in bones of Tatra chamois from the alpine zone did not differ from levels in bones of red deer and roe deer from the forest zone ($p=0.4206$). The situation is illustrated in Fig. 1.

Bones from Slovakia were additionally divided into two categories: bones of axis skeleton and bones of limbs. The Mann–Whitney U test revealed a significant difference between mercury accumulation in different bone types ($p=0.0056$) (Fig. 2).

Discussion

Mercury in bones in general

Measured concentrations of T-Hg (mg kg^{-1} dry weight) in ungulate bones were generally low. Bones are generally not a target organ for mercury accumulation (Álvarez-Fernández et al., 2021) as among heavy metals, mainly lead is accumulated in bones (Lazarus et al., 2005; Zaccaroni et al., 2008). Both forms of mercury—ionic and organic (methylmercury)—bind well to the sulfhydryl (thiol) group (Bjørklund et al., 2017; Ajsuvakova et al., 2020). However, these macromolecular structures are not found in bone. Incorporation of mercury into bone occurs through transformation into Hg_3PO_4 and HgO (Cervini-Silva et al., 2021), though mercury in the pores of bone tissue does not interfere with the bone. HgO is more soluble and not very stable, and its stability is ensured by bone minerals that prevent its dissolution in environmental conditions under the influence of moisture, temperature, and microbial activity (Cervini-Silva et al., 2021).

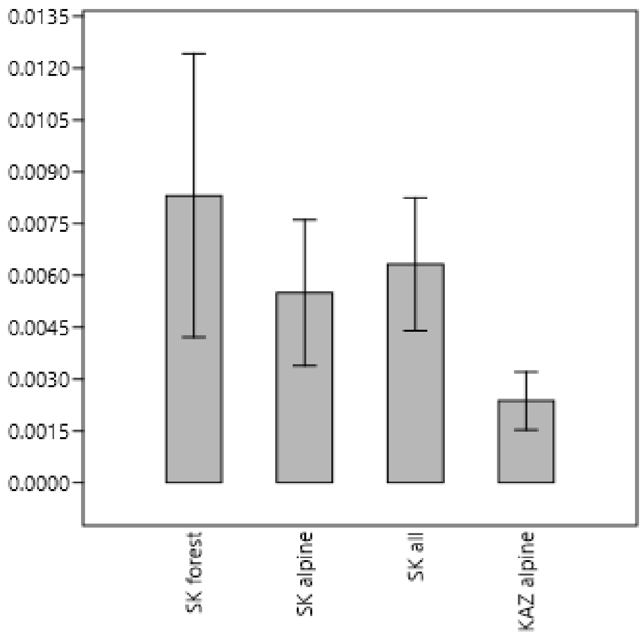

Fig. 1. Comparison of total mercury levels (mg/kg dry weight) in bones. Content of mercury in bones from animals collected in Zhongar Alatau (Kazakhstan) differed significantly from all groups of bones collected in Western Carpathians (Slovakia). Bar length: mean; whisker length: standard error; y-axis – T-Hg (mg kg^{-1} dry weight).

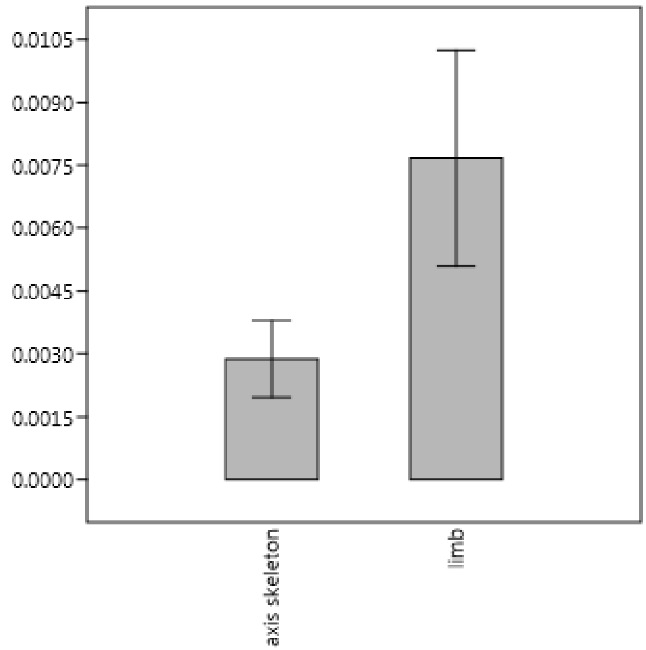

Fig. 2. Comparison of total mercury levels (mg/kg dry weight) in different bone types from Slovakia. Content of mercury in bones of axis skeleton differed significantly from bones of limbs. Bar length: mean; whisker length: standard error; y-axis – T-Hg (mg kg^{-1} dry weight).

Locality

High contaminant concentrations at high altitudes originate from emissions transported over a long distance (Bing et al., 2018; Ballová et al., 2019). High levels of metal pollution have been recorded in alpine regions (Janiga, Haas, 2019). Mountain ranges act as a direct geographical barrier to the transport of pollutants (Townsend et al., 2014). Additionally, altered meteorological conditions at higher altitudes are conducive to increased deposition. Higher frequency of rainfall (Valašková, Janiga, 2014; Tripathee et al., 2020) as well as fog and clouds (Schröder et al., 2010) increases wet deposition of soluble mercury ions (Jitaru, Adams, 2004). Strong winds (Valašková, Janiga, 2014) also contribute to dry deposition of insoluble forms by adhesion to the soil surface and vegetation (Jitaru, Adams, 2004). Concentrations of mercury detected in bank voles (Kompišová Ballová et al., 2020) and snow voles (Martinková et al., 2019) in the subalpine zone of the Tatra mountains were comparable to levels in highly polluted sites near industrial operations. It is assumed that the territory of south-eastern Kazakhstan is less affected by Hg deposition than central Europe (Travnikov, 2005). High pollution of mercury and other elements in the Western Carpathians compared to other regions was also confirmed by Maňkovská et al. (2008), when comparing concentrations in mosses from different regions of Slovakia and Norway. Mercury concentrations were 8.3–26.3 times higher than that in Norway. Metal pollution originating from long-range transport was also observed for other metals. The bones of snow voles collected in the Tatra Mountains contained significantly more lead than the bones of voles from the Bulgarian Rila and Vitosha mountains (Janiga et al., 2016) as well as bones of their Kyrgyz ecological equivalent the silver mountain vole (*Alticola argentatus*) and narrow-headed vole (*Microtus gregalis*) (Ballová, Janiga, 2018).

In accordance with previous published reports and studies, our results show that T-Hg concentrations in bones collected in Zhongar Alatau were significantly lower than those in the Western Carpathians. However, the tendency of higher contamination of forest habitats compared to the alpine zone (as described below) biases interpretation of the results. The group of Slovak animals was not homogenous according to habitat and included both alpine and forest species. Comparison of the Kazakh group with Tatra chamois revealed a difference with a p-value higher than that in previous analysis, but still below the threshold of significance. This result supports the assumption that the high-elevation zone of Zhongar Alatau and the Slovak alpine zone (localities of similar altitude) differ in their level of contamination by mercury.

Habitat

When comparing Hg concentrations between the bones of Tatra chamois from the alpine zone and bones of roe deer and red deer from woodland habitats, no difference was demonstrated. On average, concentrations were higher in bones from the forest habitats, although the difference was not significant.

It has been suggested that habitat or ecotype may be one factor influencing the amount of Hg found in ungulates. Comparisons of the effect of habitat on Hg and other metal/nutrient concentrations are present in the available literature. Demesko et al.

(2019) compared trace element content in roe deer in both field and forest ecotypes. It has been shown that trace element concentrations were lower in the forest ecotype, which may be explained through anthropogenic pollution of the field habitat, type of preferred food, home range size, level of sociality, differences in behavior, and antipredator strategy. Similar studies by Tajchman et al. (2020, 2021) investigated the abundance of micro- and macro-elements and toxic elements (without Hg) in bone and bone marrow of wild and farmed ecotypes of red deer. Whereas most nutrients reached higher levels in farmed animals, toxic elements varied depending on the element and tissue type. The higher abundance of macro-nutrients in bones of wild animals is related to a higher dietary diversity, as wild animals are able to forage for food that contains higher mineral content. Nawrocka et al. (2020) compared Hg in organs in game animals and livestock in Poland. Meat and liver of game animals were found to contain higher concentrations of Hg than that of domesticated animals. It was also observed that different habitats can lead to differences in mercury accumulation in animal bodies as a result of natural presence and availability of mercury in the environment (Komov et al., 2017), anthropogenic pollution (Dahmardeh Behrooz et al., 2022), food availability (Dainowski et al., 2015), and behavior and lifestyle (Skibniewska, Skibniewski, 2023).

Pollution of alpine habitats by metals from the atmosphere (Patra, Sharma, 2000; Bing et al., 2018; Ballová et al., 2019, Janiga, Haas, 2019) is arguably the cause of higher concentrations in the bones of chamois living in alpine habitats particularly because their diet consists of long-lived alpine plants that accumulate more atmospheric mercury (Kompišová Ballová et al., 2021). However, a nonsignificant result did not support this assumption. A case for higher concentrations in forest habitats can be made due to the higher deposition rate under forest canopies than in open areas (Mowat et al., 2011). Forest cover and the presence of tree vegetation trap atmospheric mercury in leaves (Bing et al., 2018; Tripathee et al., 2020). Gaseous elemental mercury from the atmosphere is taken up by leaf stomata (Townsend et al., 2014). Although needles accumulate mercury more slowly than leaves of deciduous trees, long-lived needles are exposed to atmospheric mercury for longer periods of time (Blackwell, Driscoll, 2015). Therefore, high-elevation coniferous stands accumulate more Hg than deciduous forests (Gerson et al., 2017; Townsend et al., 2014). Ma et al. (2021) investigated the relationship between soil and small mammal concentrations with increasing elevation and also showed that mammal hair concentrations are highest in the coniferous zone. Three different sites—the Whiteface Mountains (Gerson et al., 2017; Blackwell, Driscoll, 2015), the Catskill Mountains (Townsend et al., 2014), and the Eastern Himalayas (Ma et al., 2021)—show the highest Hg concentrations and deposition rates in the coniferous zone despite the varying range of vegetation stages.

Bone type

As shown in our research, the bones of the limbs showed higher T-Hg concentrations on average than the bones of the head. Emslie et al. (2019) hypothesize that soil contaminants can get into the bone through the pores in solid state or in the form of solutions. It has also been hypothesized that higher concentrations of mercury exist in bones near the abdominal area due to decomposition of tissues such as the kidney and liver, which are target organs for Hg and whereby Hg translocated to the nearby pelvic and rib bones (Álvarez-Fernández et al., 2021, 2022). However, this is only true if the bones were buried where there was an opportunity for soft tissue decomposition. In our case, postmortem translocation from soft tissue to bone is unlikely as the bones were not buried. This may explain why the axial skeleton bones collected by us did not contain the expected high concentrations. It is assumed that these animals were either hunted by predators or died naturally and soft tissues became a food for scavengers. Vertebrate scavengers translocate nutrients and redistribute them across the landscape, while decomposers among invertebrates and microorganisms leave nutrients near the carcass (Peers et al., 2021; Butler-Valverde et al., 2022).

The reason for varying accumulation in different bone types may also be due to the different representation of trabecular (spongy) and compact (cortical) bone tissue. Higher concentrations of Hg in trabecular tissue have been found by Rasmussen et al. (2013, 2017) in archeological bones, where concentrations can in some cases be up to 40-fold the concentration in compact tissue. As bone is not a target organ for Hg (Álvarez-Fernández et al., 2021), the Hg in this tissue likely was absorbed from soft tissues that were in contact with the trabecular bone during life, such as blood, fat, bone marrow, blood vessels, and nervous tissue. After death and decomposition of these tissues, Hg was deposited in the adjacent trabecular bone (Rasmussen et al., 2013).

Conclusion

We compared mercury concentrations in the bones of wild ungulates at two geographically distant sites of similar elevation and habitat. The territory of southeastern Kazakhstan was shown to be less polluted by mercury than Western Carpathians. We suggest that this is mainly due to geographical isolation from anthropogenic pollution sources. By contrast, the Western Carpathian Mountains in central Europe capture large amounts of contaminants transported by the atmosphere from densely populated and industrial areas in Europe. It has been shown that with the use of precise determination technology, bones can be a suitable material for the study of mercury contamination of biota. It is possible to use this method to identify other potentially clean areas that are less affected by pollution with mercury and other pollutants.

Hg accumulates more in the bones of the limbs than in the bones of the axial skeleton. We could not sufficiently explain the reason for this difference. The consideration of possible causes is a matter of discussion, not the main objective of the study.

On average, higher concentrations were recorded in the bones of species from the forest habitat, which agrees with other studies of a similar theme. However, it should be emphasized that different habitats with different altitudes are inhabited by different species. According to the known ecology of the species, we do not expect too much overlap in ecological requirements between *Rupicapra rupicapra tatrica* and Cervidae at the study site. However, we cannot exclude the possibility of physiological variance of the studied species causing differences in mercury accumulation that are not related to contamination of their food and environment. The comparison of mercury contamination in forest and alpine habitats using a single species of herbivorous ungulate is difficult due to the absence of a species that would be able to inhabit both habitats while avoiding vertical migration.

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