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Non-destructive mercury exposure assessment in the Brandt's hedgehog (*Paraechinus hypomelas*): spines as indicators of endogenous concentrations

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Abstract

Due to its persistence, bioaccumulation characteristics and toxicity, environmental contamination with mercury (Hg) is of high concern for human health, living organisms and ecosystems, and its biological monitoring is highly relevant. In this study, the levels of total Hg were measured in organs, tissues and spines of 50 individuals of Brandt's hedgehog collected in Iran in 2019. The Hg median levels in kidneys, liver, muscle, and spines were 156, 47, 47 and 20 ng/g dry weight, respectively. The results showed a significant positive correlation between the levels of Hg in kidneys and liver (r = 0.519; $p < 0.01$) and in spines and muscle (r = 0.337, $p < 0.01$) and kidneys (r = 0.309, $p < 0.05$). Significant differences ($p < 0.05$) in Hg levels in organs and tissues were also observed depending on the sex, weight, length, and age of the individuals. In addition, the median levels of total Hg in kidneys of Brandt's hedgehogs from an agricultural ecotype (mean 179±65) were significantly higher ($p < 0.05$) than those collected from a forest ecotype (mean 122±50), suggesting that the habitat could have a significant impact on animal contamination.

Keywords: Mercury, Iran, Habitat ecotype, tissues.

Introduction

Mercury (Hg) is an non-essential element which can cause toxic effects in humans and biota when it enters the body, mostly through ingestion, inhalation and dermal absorption, and reaches concentrations above a certain threshold (Pastorinho and Sousa 2020). Natural sources of Hg are responsible for about half of atmospheric emissions, while the remaining half derives mostly from anthropogenic sources, such as chemical industry emissions, smelting and melting of other metals (e.g. gold), wastewater treatment, improper disposal of certain products, and the use of pesticides and fertilizers (Smart and Hill 1968; Mortvedt 1995; Navarro et al. 1996; Wagner-Döbler 2003; Yasuda et al. 2004; Zheng et al. 2007; Zhong et al. 2018; Tang et al. 2018, 2020; Sun et al. 2019b; Wang et al. 2019). Due to its known toxicity, bioavailability and bioaccumulation potential, Hg environmental pollution has caused a growing worldwide concern (Gutiérrez-Mosquera et al. 2021). When reaching aquatic ecosystems, inorganic Hg can be methylated to methylmercury (MeHg) by the action of microorganisms and bioaccumulate into the food chain, where it can cause severe damage to the biota and eventually to humans, including developmental and neurological health issues (Nogara et al. 2019; Gutiérrez-Mosquera et al. 2021).

Assessing Hg environmental contamination through appropriate monitoring programs is thus paramount to preserve the value and biodiversity of ecosystems and evaluate the need for potential remediation actions. Such monitoring programs often include the analysis of various tissues or organs of animals, including fish, birds, and mammals, considered suitable bioindicators of environmental Hg pollution (Singh et al. 2017; Sun et al. 2019a; Dahmardeh Behrooz and Poma 2020; Poma et al. 2020). While several studies have focused on measuring Hg levels in animal organs, such as liver and kidneys (Dip et al. 2001; Gamberg et al. 2005; Horai et al. 2006), fewer studies are currently available on investigating Hg levels in mammalian hair, although this matrix has been praised for its ethical and practical advantages (May Junior et al. 2018; Becker et al. 2018; Crowley and Hodder 2019; Martinková et al. 2019; Dahmardeh Behrooz and Poma 2020; Kosik-Bogacka et al. 2020). In particular, hair i) can be easily collected, stored and transported, ii) can be sampled in a non-invasive manner, allowing the monitoring of threatened and/or endangered species, iii) can incorporate and retain chemicals through the hair follicle, iv) allows the elimination of toxic elements from the body when it grows, and v) can be a good indicator of the amount of Hg in the body, showing high correlation between the metal concentrations in hair and in other organs (Crowe et al. 2017; Rendón-Lugo et al. 2017; de Castro and de Oliveira Lima 2018; Yamanashi 2018; Eyrikh et al. 2020).

Among other animals, hedgehogs are considered suitable bioindicators of (local) Hg environmental pollution because they have a small home range, limited migration rate, long life span, and they are often found living near human residential areas and agricultural lands (D'Havé et al. 2005, 2006a, b). In addition, hedgehogs are a mammalian insectivorous species, feeding mostly on beetles, caterpillars, earthworms and slugs, organisms at the bottom of the food chain and in close contact with the soil (Hendriks et al. 1995; Reinecke et al. 2000). Finally, positive relationships have been previously found between metal concentrations in hair, spines, and organs of hedgehogs (D'Havé et al. 2005). The spines, modified hairs with a thick, hard, outer tube of keratin which mostly serve as defense from predators, may thus have the same potential as hair in assessing the metal body burden of the organism.

The aim of this study was to assess the concentrations and correlations of total Hg in the organs (liver, kidneys), tissues (muscle), and spines of 50 Brandt's hedgehogs (*Paraechinus hypomelas*) collected from the Sistan region of Iran. The potential of spines as a non-invasive biological matrix to assess Hg pollution in terrestrial ecosystems and the potential differences in contamination related to the habitat of the selected species were also investigated.

2. Materials and methods

2.1. Collection of samples

Hedgehog samples were collected during summer 2019 from roads passing through forested and agricultural areas in the Sistan region of Iran (Fig. 1). For 30 days, researchers and local volunteers visited each morning selected locations along the road screening for hedgehogs killed in car accidents during the previous night. The least damaged individuals (meaning with bodies left relatively intact) were collected for the study. Length and weight of each individual was recorded, samples were then labelled, placed into zip-lock plastic bags, and stored at -20 °C for transportation. Once at the laboratory, sex and age were determined following available protocols (Reeve and Lindsay 1994; Rautio et al. 2010). Each individual was then dissected, the liver, kidneys and muscle tissues were removed and stored at -20 °C pending analysis, while the spines were carefully cut from the body using metal scissors (pre-cleaned with deionized water and acetone) and kept at room temperature pending analyses (Dahmardeh Behrooz et al. 2020). Due to the limited, but still present, damage of the individuals following car accidents, hair samples were not considered suitable for collection and analysis.

2.2. Sample preparation and analysis

Spine samples were first washed with tap water and soft detergent, followed by three rounds of distilled water to remove any detergent residue, dirt particles, and other superficial impurities, and finally with acetone, following the same protocol in use for the determination of Hg in hair samples (Solgi and Ghasempouri 2015). The spine samples were then dried at room temperature in a dust-free atmosphere and fine-cut with pre-cleaned scissors to resemble powder. Liver, kidney and muscle samples were dried at 60 °C for 92 h and each powdered in a Chinese mortar to obtain a homogeneous matrix.

Spines (~ 25 mg) and dried organ and tissue samples (~ 50 mg) were weighed and immediately analyzed using an AMA 254 Mercury analyzer (Leco Corporation Agilent Tech, CA, USA), for which no previous chemical digestion step is requested. Ultrapure oxygen was used as a carrier gas with an inlet pressure of 250 kPa and a flow rate of 200 mL/min. Each sample was analyzed in triplicate.

2.3. Quality assurance and quality control

Instrument calibration was performed with a NIST-traceable Hg std solution (AccuTrace Single Element Standard; AccuStandard Inc., New Haven, CT, USA). Seven replicate analysis of standard reference materials SRM 1633b (Constituent Elements in coal fly ash), SRM 2709 (San Joaquin Soil Baseline Trace Element Concentrations), and SRM 2711 (Montana II soil) were used for checking the reliability of the analysis. Accuracy of SRM measurements ranged between 86% and 111%, with a relative standard deviation (RSD) $<$ 15% (Table 1). To prevent carry-over effect, at least one procedural blank was analyzed after three replicates of the same sample. The method detection limit (LOD) was estimated at 0.3 ng/g dry weight (dw) for all considered matrices. The limit of quantification (LOQ) of the proposed method were measured in blank samples and calculated by considering as 3 x procedural blank and assessed at 1 ng/g dry weight (dw). Due to the low concentration of mercury in the tissues, the device was set to low calibration curve after a few repetitions.

2.4. Statistical analysis

Statistical analysis was carried out with the SPSS software (Version 16.5). Data were tested for normality using a Kolmogorov-Smirnov test and found normally distributed after log-transformation (log 10). After normal distribution and homogeneity of variance of mercury levels in the samples, parametric statistics were employed. During statistical analysis, non-detects were substituted with zero (<LOQ = 0, i.e. lower bound, LB). An independent t test was used to assess possible differences in hedgehog tissue concentrations depending on gender and ecotype. Spearman's rank correlation coefficients were used to test for correlations among various Hg levels in the different tissues. Significant differences were assumed at $p < 0.05$.

3. Results and discussion

3.1 Mercury concentrations in Brandt's hedgehogs

Mercury levels of Brandt's hedgehogs [median; mean ± SD] ranged from 6 to 270 ng/g dw [156; 150 ± 65 ng/g dw] in kidneys, from 2 to 264 ng/g dw [47; 66 ± 61 ng/g dw] in liver, from 3 to 108 ng/g dw [47; 44 ± 26 ng/g dw] in muscles, and from 1 to 94 ng/g dw [20; 27 ± 20 ng/g dw] in spines (Table 2).

A previous study has shown that mercury concentrations in bear hair samples above 6,000 ng/g dw would likely cause observed subclinical neurological effects in the animals (Dietz et al. 2011). Even more so, such neurological effects have been noticed also in mink, when the concentrations of mercury in the hair of this animal were measured up to 30,000 ng/g dw (Basu et al. 2007). According to previous studies, a mercury concentration of 1100 ng/g in liver and kidneys is considered a threshold level for serious health effects in wild mammals (Eisler 1987), while levels of mercury up to 125,000 ng/g dw in kidney tissues of carnivorous mammals were showed to cause fatal poisoning (Beyer and Meador 2011). In addition, 30 mg/g Hg in mammalian liver and kidney tissues is considered as an intoxication threshold, with levels up to 69 mg/g reported in the kidneys of wild and laboratory mammals whose deaths was attributed to mercury poisoning (Wren 1986; Lord et al. 2002; Rezayi et al. 2011). Finally, the U.S. EPA set the lowest guideline value for mercury in human hair at 1000 ng/g dw (Dietz et al. 2011). The concentrations of Hg measured in the organs and spines of the Brandt's hedgehog specimens analyzed in this study were considerably lower than all above-mentioned values, suggesting the absence of toxic effects for the considered wildlife.

The mean Hg levels in the liver of Brandt's hedgehogs (66 ng/g dw or 198 ng/g ww) (Rezayi et al. 2011)) were generally higher than the average mercury levels measured in liver tissues from the European hedgehog (*Erinaceus europaeus*), fox (*Vulpes vulpes*), porcupine (*Hystrix cristata*), stone marten (*Martes foina*), and badger (*Meles meles*) collected from the Italian Province of Pesaro and Urbino (Alleva et al. 2006), and higher than the multi-organ and hair Hg concentrations in Russian wild boars (*Sus scrofa*) (Eltsova and Ivanova 2021) (Table 3). Average Hg concentrations in the organs and spines of the Brandt's hedgehogs were instead comparable to or lower than those measured in tissues and hair of bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*) collected in the UK (Bull et al. 1977), and golden jackal (*Canis aureus*) from the region of Mazandaran, Iran (Malvandi et al. 2010) (Table 3). Finally, average Hg levels in the tissues and spines of the Brandt's hedgehogs were lower than those measured in raccoons (*Procyon lotor*) in the Polish Warta Mouth National Park (Lanocha et al. 2014), Arctic foxes (*Vulpes lagopus*) from inland and coastal regions of Iceland (Treu et al. 2018), American martens (*Martes americana*) and northern short-tailed shrew (*Blarina brevicauda*) from USA (Witt et al. 2020)(Talmage and Walton 1993) (Table 3). The overall mercury contamination of the Brandt's hedgehogs collected from the Sistan region of Iran resulted generally lower than of animals collected near known contamination sources, but nonetheless higher than levels in animals collected where no sources of Hg contamination have been reported (Table 3). This suggests that the habitat of the Iranian hedgehogs is affected by mercury presence, likely deriving from the application of chemical fertilizers and pesticides.

3.2. Ecological factors affecting mercury levels

Several research studies showed that mercury levels in animal tissues and organs are potentially influenced by physiological and ecological factors, such as sex, age, size, feeding strategy, and habitat (Malvandi et al. 2010; Bilandžić et al. 2010; Zarrintab and Mirzaei 2017; Treu et al. 2018; Eyrikh et al. 2020).

In this study, the females presented significant lower Hg concentrations than males ($p < 0.05$) in the analyzed kidneys and muscle tissues (Table 2), suggesting that the mercury burden in the body of female hedgehogs might be reduced by transfer to the fetus through the placenta and to offspring during lactation, as widely described for other mammals (Yoshida et al. 1994; Frodello et al. 2000). Previous research also indicated that the levels of Hg in an organism are expected to increase with age and size, mostly due to the slower removal of this metal from the body and/or the longer time of exposure in older individuals (Braune et al. 2015). Also, in this study, the levels of Hg in selected hedgehog organs correlated with weight, length, and age. A significant positive correlation was observed between the levels of mercury in liver and kidney tissues and weight ($r = 0.460$, $p < 0.05$, $r = 0.295$, $p < 0.05$, respectively), between the levels of mercury in kidneys, muscle and spines with length ($r = 0.471$, $p < 0.01$; $r = 0.291$, $p < 0.05$; $r = 0.342$, $p < 0.05$, respectively), and between the levels of mercury in kidneys, liver and spines with age of the animals ($r = 0.530$, $p < 0.01$; $r = 0.334$, $p < 0.05$; $r = 0.362$, $p < 0.01$, respectively) (Table 4). As expected, the age of the animals positively correlated with their weight and length ($p < 0.01$), highlighting the positive relation between age and mercury accumulation in the animal tissues (Ben-David et al. 2001; Gerstenberger et al. 2006). The average age of hedgehogs analyzed in this study was 2.4 years, about one third of this species life expectancy, likely implying that mercury had enough time to accumulate in the individuals' internal tissues.

To investigate if the habitat of the animals could also have influenced their contamination, the levels of mercury in organs and spines of Brandt's hedgehog specimens collected from an agricultural ecotype (n=25) were compared with those from a forestry ecotype (n=25). Median Hg levels in kidneys of hedgehogs from the agricultural ecotype (190 ng/g dw) were significantly higher ($p < 0.05$) than those from the forestry ecotype (126 ng/g dw) (Table 2), while no significant differences were observed comparing the Hg concentrations in the other tissues. The overall higher mercury levels of Brandt's hedgehogs collected from the agricultural ecotype could be likely associated with human presence in this area and the use of mercury in chemical fertilizers and pesticides (Benhaiem et al. 2008; Demesko et al. 2019). To date, urbanization and human-related land alteration (e.g., intensive agricultural activities) have been often associated with increasing metal contamination levels, including As, Cd, Cu, Pb, and Hg, in a wide variety of wildlife (Orlowski et al. 2008; Bilandžić et al. 2010; Flache et al. 2015). In this study, the higher mercury concentrations in Brandt's hedgehogs collected from the agricultural ecotype could be due to the direct absorption of contaminants from the soil, given that this species has a small habitat surface and that farmers in this area use pesticides that might contain. Research has shown that, among small mammals, insectivores are more exposed to environmental toxins than herbivores, which may be due to the direct absorption of contaminants from the soil and their placement in the middle of the food chain (D'Havé et al. 2006b).

Our results strengthen the hypothesis that a higher bioaccumulation of harmful substances of anthropogenic origin in wild animal populations can be driven by the proximity of human settlements (Demesko et al. 2019; Dahmardeh Behrooz et al. 2020).

3.3. Correlations between mercury levels in different tissues

Significant correlations were observed between Hg concentrations in the analyzed hedgehog tissues (Figure 2 and Table 4). Hg levels in liver tissues were significantly correlated with those in kidneys (r = 0.519, $p < 0.01$), followed by spines with kidneys (r = 0.337, $p < 0.01$) and muscles (r = 0.309, $p < 0.05$), respectively. This outcome agrees with the results of other studies in mammals, suggesting that the levels of mercury measured in hair and spines reflect those in organs and soft tissues (Ikemoto et al. 2004; Dainowski et al. 2015; Treu et al. 2018), and supports the use of non-destructive tissues for the monitoring of mercury environmental pollution (Dahmardeh Behrooz and Poma 2020; Dahmardeh Behrooz et al. 2020).

The stronger correlation found between the levels of mercury in liver and kidney, rather than between spines and organs/tissues, could be mostly attributed to the active Hg metabolism in these two organs which are directly connected through the bloodstream (Treu et al., 2018; Boening, 2000). The reabsorption of Hg via enterohepatic recirculation in the animal body, as mentioned by Boening (2000), can thus explain the strong correlation observed between mercury levels in liver and kidney of the Brandt's hedgehog. On the other hand, the absence of a significant correlation between spine and liver Hg levels could be due to the role played by factors such as age, sex, sampling location and the species-specific detoxification capacity of the Brandt's hedgehog. Finally, a possible residual external contamination with Hg on animal hair and spines, even after washing steps, has been suggested as a possible additional source of contamination variability, potentially affecting the body-burden relationships (Morton et al. 2002; Li et al. 2008).

Since the specific kinetics of mercury accumulation and detoxification in organs and hair in different animal species are not fully understood yet, there is the need to further investigate Hg complex metabolic transformation processes, especially in terrestrial mammals. On the other hand, the strong correlation between the levels of mercury in the liver and kidneys and between hedgehog spines and kidney and muscle tissues suggests that Brandt's hedgehog spines can be a valuable non-invasive tool for environmental measurement and monitoring of Hg environmental pollution, but caution is advised when translating the outcomes deriving from this study to other species.

Conclusions

In this study, the levels of mercury were measured in Brandt's hedgehog organs, muscle tissues and spines. The results showed a significant positive correlation between the levels of mercury in Brandt's hedgehog spines and muscle and kidney tissues, suggesting that hedgehog spines can be used as a non-destructive tissue in the monitoring of mercury environmental pollution. Also, living near human residential areas and agricultural lands could have caused a significant increase in levels of mercury in hedgehog tissues. The results of this study showed that also physiological parameters, like sex, size and age, can significantly affect the Hg pollution burden of the animals. These outcomes set scientific basis for the introduction of the Brandt's hedgehog and its spines as an environmental indicator for measuring metal pollution in terrestrial ecosystems.

Author contribution: RDB - Conceptualization, Formal analysis, data curation, investigation, Writing – Original Draft preparation; GP and MB - methodology, Writing - Review & Editing. All authors have read and agreed to the current version of the manuscript.

Availability of data and materials: The data and materials for this work are available upon request.

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Ethics approval and consent to participate: All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee University of Zabol with reference number 004.1399.REC.UOZ.IR.

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Figure 1. Sampling location roads and ecotypes: 1, forest and 2, agricultural.

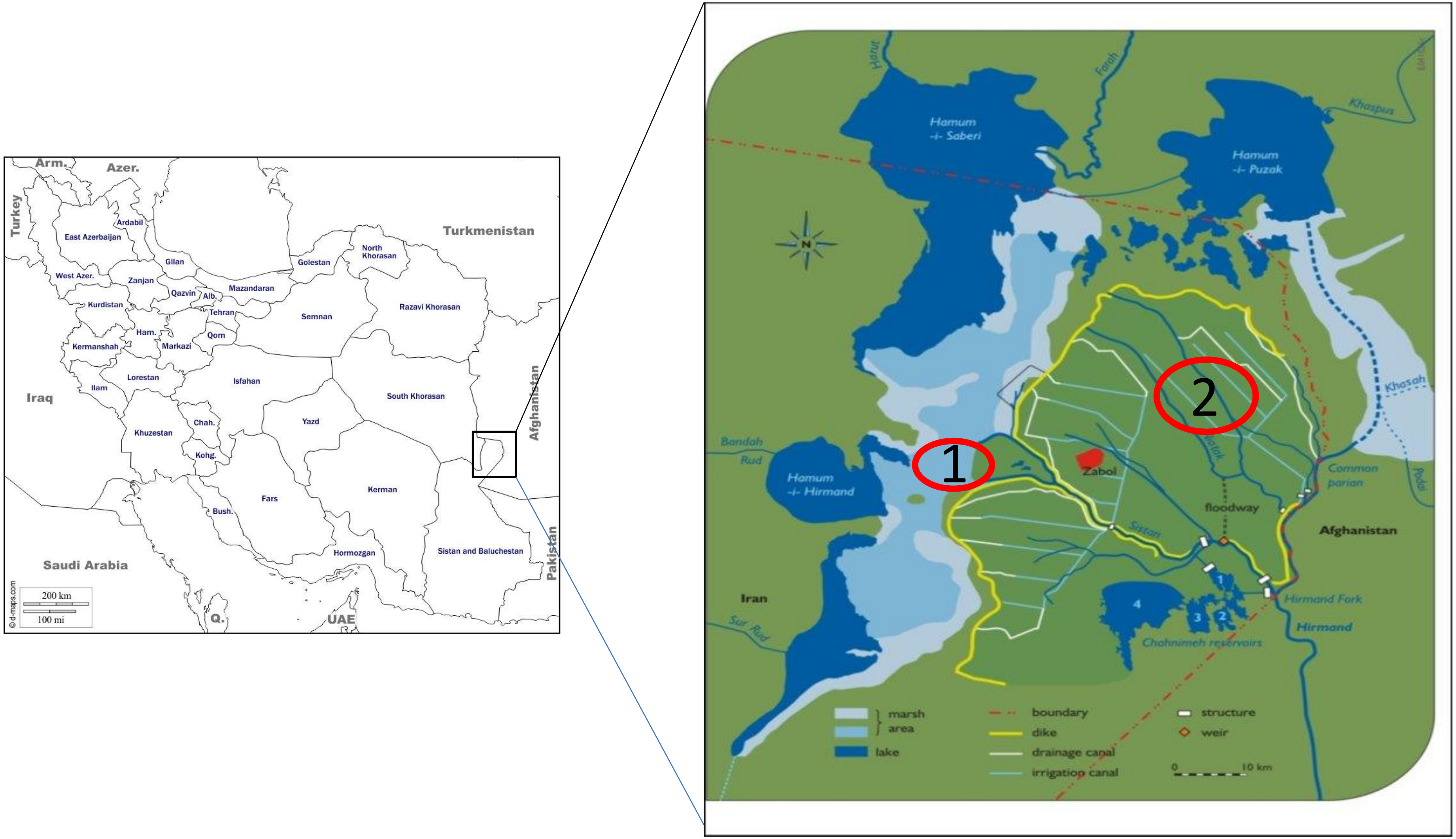

Figure 2. Correlation between total mercury concentrations (ng/g dw) in organs, tissues and spines from the Brandt's hedgehogs.

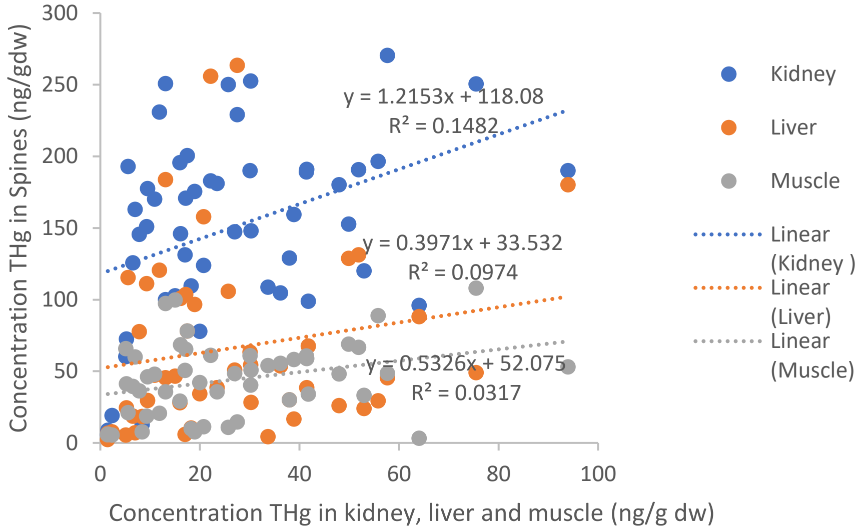

Table 1. Results of quality assurance procedure for mercury analysis (μg/g). NIST: National Institute of Standard and Technology

SRM	Certified value	Our results	Accuracy
NIST-1633	0.141	0.142	100.7
NIST-2709	1.400	1.558	111.2
NIST-2711	6.250	5.411	86.57

Table 2. Physiological parameters and descriptive statistics of total Hg (ng/g dw) in organs and spines from hedgehog individuals. * $p < 0.05$.

		Weight (g)	Length (cm)	Age (year)	Kidney	Liver	Muscle	Spines
Total (n=50)	Mean±SD	448±89	22±2	2.4±2	150±65*	66±61	44±26	27±20
	Median	468	23	2	156	47	47	20
	Minimum	102	11	< 1	6	2	3	2
	Maximum	551	26	6	270	264	108	94
Sex								
Male (n=30)	Mean±SD	465±42	23±1		159±51*	70±70	49±22*	29±19
	Median	466	23		156	47	49	25
	Minimum	386	20		60	4	10	5
	Maximum	551	26		270	264	108	76
Female (n=20)	Mean±SD	421±129	22±3		138±81*	60±46	37±30*	23±22
	Median	470	22		159	47	38	18
	Minimum	102	11		6	2	3	1.5
	Maximum	550	26		253	180	100	94
Ecotype								
Forest (n=25) (15 male/10 female)	Mean±SD	436±89	22±3		122±50*	59±60	43±26	23±17
	Median	453	22		126	47	48	19
	Minimum	102	11		6	2	3	1
	Maximum	534	25		197	256	100	64
Agriculture (n=25) (14 male/11 female)	Mean±SD	460±89	23±2		179±65*	74±63	45±26	31±23
	Median	481	23		190	49	46	26
	Minimum	150	17		13	7	6	2
	Maximum	551	26		270	264	108	94

Table 3. Average Hg concentration (ng/g dw) in different tissues of Brandt's hedgehog and other mammals from previous studies.

English name	Scientific name	Location	Year	Liver	Kidney	Muscle	Hair	Ref.
Wood mice (n=6)	*Apodemus sylvaticus L.*	UK. Around a chlor-alkali industrial area	1974	230[a]	520[a]	980[a]	780[a]	(Bull et al. 1977)
Bank vole (n=7)	*Clethrionomys glareolus*			150[a]	350[a]	280[a]	910[a]	
Shorttail shrew (n=8)	*Blarina brevicauda*	Oak Ridge, USA. Recorded Hg polluted region	1986-1987		38800[b] (12933[a])			(Talmage and Walton 1993)
European hedgehog (n>5)	*Erinaceus europaeus*	Urbino–Pesaro province, Italy. No reported source of Hg contamination	1994-1995	60[b] (20[a])				(Alleva et al. 2006)
Fox (n>5)	*Vulpes vulpes*			30[b] (10[a])				
Porcupine (n>5)	*Hystrix cristata*			10[b] (3[a])				
Stone marten (n>5)	*Martes foina*			110[b] (20[a])				
Badger (n>5)	*Meles meles*			180[b] (37[a])				
Golden jackal (n=21)	*Canis aureus*	Mazandaran, Iran. No reported source of Hg contamination	2007-2008	53[a]			178[a]	(Malvandi et al. 2010)
Raccoon (n=24)	*Procyon lotor*	Warta Mouth National Park, Poland. Presence of coal mining and metallurgic industries	2009-2011	2990[a]	2070[a]	500[a]		(Lanocha et al. 2014)
Fox	*Vulpes lagopus*	Iceland	2011-2012	8240[b] (2747[a])	6330[b] (2110[a])		7940[b] (2647[a])	(Treu et al. 2018)
American marten (n = 40)	*Martes americana*	Michigan, USA. Recorded Hg polluted region	2013-2014	344[a]	922[a]		1228[a]	(Witt et al. 2020)
Wild boar (n=25)	*Sus scrofa*	Russky Sever National Park (Russia). No reported source of Hg contamination	2014-2019	7[b] (2.3[a])	79[b] (26.3[a])	4[b] (1.3[a])	42 (14[a])	(Eltsova and Ivanova 2021)
Brandt's hedgehog	**Paraechinus hypomelas**	***Sistan region, Iran***	***2019***	***66*** [a]	***150*** [a]	***44*** [a]	***27*** [a] ***(spines)***	***This study***

a: concentration in ng/g dw
b: concentration in ng/g ww

Table 4. Spearman's rank correlation between total mercury concentrations (ng/g dw) in organs, tissues and spines from the Brandt's hedgehogs (n = 50). * = *p* < 0.05, ** = *p* < 0.001.

	Kidney	Liver	Muscle	Spines	Weight	Length	Age
Kidney	1						
Liver	0.519^{**}	1					
Muscle	0.24	0.074	1				
Spines	0.377^{**}	0.274	0.309^{*}	1			
Weight	0.460^{**}	0.295^{*}	-0.077	0.193	1		
Length	0.471^{**}	0.2	0.291^{*}	0.342^{*}	0.487^{**}	1	
Age	0.530^{**}	0.334^{*}	0.255	0.362^{**}	0.421^{**}	0.847^{**}	1