

Mercury bioaccumulation in hair and skin of arctic foxes (*Vulpes lagopus*) and silver foxes (*Vulpes vulpes*) in rural and urbanized region

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ABSTRACT: Mercury bioaccumulation in hair and skin of silver and arctic foxes farmed in typically rural and urbanized regions (Wielkopolskie Voivodship, Poland) was assessed. Hair and skin samples were collected and analyzed for total Hg content using atomic absorption spectrometry. Hairs and skin of foxes farmed in the rural region accumulated higher amount of Hg compared to animals from the urbanized one. Species effect (lower Hg concentration in *V. lagopus*) was noted, females having higher accumulation compared with males. The highest Hg content was observed in hairs of *V. vulpes* females in the rural region (0.207 mg/kg on average), and in skin of *V. lagopus* females (0.0082 mg/kg on average). Highly significant correlation ($r = 0.796$) was noted between Hg content in skin and hairs of farm foxes. The present study confirms the previous finding that non-invasively collected hair samples are a good tool applicable in evaluating heavy metal load of different environments.

Keywords: farm; Canidae; hair coat; environment; mercury level; Poland

INTRODUCTION

Mercury has strong chemical and biological activity, as well as changeable forms (liquid and gaseous). It is not essential in life processes of plants, animals, and humans, being considered a strong environmental toxin, especially in its organic form, like methyl-mercury. Toxic activity of Hg involves disorders in enzymatic apparatus functioning, and changes within phosphate DNA bonds, which is connected with its further mutagenic, embryotoxic, and teratogenic activity (Schoeman et al. 2009).

Mercury appears to be an important factor inducing a range of metabolic disorders and diseases, including neurological, nephrological, immunological,

cardiological, motoric, fertility, and even genetic ones (Zahir et al. 2005; Virtanen et al. 2007). The source of Hg emission into the environment is combustion of oil and coal products, ferrous and non-ferrous metals smelting industry, industrial processes applying Hg and its compounds, cement production, wastes combustion, and others (Szyrkowska et al. 2003). Mercury is commonly detected in environment and living organisms, including Arctic and Antarctic animals (Hoekstra et al. 2003; Dommergue et al. 2010; Bocharova et al. 2013).

It is estimated that total annual Hg emission both from natural and anthropogenic sources was 4800–8300 Mg per year at the beginning of the 21st century (UNEP Chemicals, Report No. 54790-01, www.chem.unep.ch. 2002).

In Poland, according to various scenarios (BAU, POT, DEG), its amount was estimated at a level of 6.2–17.4 Mg in 2010 (Glodek et al. 2010). Prognoses of Hg emission by ten European countries with the highest emission assume reduction to 179.6 Mg/year (BAU scenario) or 64.4 Mg/year (DEG scenario) by 2020 (Pacyna et al. 2006).

Mercury is easily transferred via respiratory or alimentary tract from an environment to plant and animal organisms, which may locally lead to an increased accumulation of this toxic metal in animal origin products.

Animal skin products are good indicators of contamination with mercury. The assimilated content of this toxic element, both from diet and air, may be assessed based on an analysis of hair, coat, bristle, wool or feathers as well as skin and different organ samples (Dey et al. 1999; Park et al. 2005).

There are some data concerning Hg content in arctic and red foxes organs in Poland as well as worldwide. For example, high Hg content in kidneys (5.11 mg/kg dry matter (DM)), in liver (4.52 mg/kg), and in muscles (1.56 mg/kg) was noted in juvenile *V. vulpes* inhabiting Mielin Island in North-West Poland (Kalisinska et al. 2009). Low Hg concentration in bones (0.002 mg/kg DM on average) was revealed in other study on *V. vulpes* (Lanocha et al. 2012). These authors claim also that the tissues and organs of free living *V. vulpes* are good bioindicators of environment pollution with Hg and other toxic metals like e.g. Cd or Pb. In arctic fox (*Vulpes lagopus* formerly known as *Alopex lagopus*) in Canada, mean Hg content observed in liver was 0.14 µg/g wet weight (Hoekstra et al. 2003), while in the USA (Alaska) in kidneys it was even 1.59 mg/kg DM (Dehn et al. 2006). Other data concerning Hg content in organs and tissues of farm and wild foxes in various regions of the world were reported by Kalisinska et al. (2012). There are however only sparse data of this toxic element content in hair coat or raw skin of both fox species. For example, Bocharova et al. (2013) determined Hg content at the level of 0.69–27.08 (10.58 on average) mg/kg DM in hair of arctic foxes from Iceland, and of 4.21–18.34 (10.42 on average) mg/kg DM on the Commander Islands.

The aim of the study was to assess Hg accumulation in hair and skin of foxes farmed in typically rural and urbanized (suburban) regions in order to determine possible differences between the examined fox species.

MATERIAL AND METHODS

Animals and diets. The research material was derived from 8-month-old common foxes (*V. vulpes*) of silver variety and 6-month-old arctic foxes (*V. lagopus*) from two farms in Central-West Poland (Wielkopolska district). The first farm is located in a typically rural region, while the second one in the suburban, urbanized area. The foxes were housed in groups in cages under a roof with uncontrolled air inflow. All foxes were fed *ad libitum* with the same fodder that was changed once during the whole rearing period (Table 1). The mixture in form of homogeneous pulp was preserved with sodium pyrosulfate (2 kg/1000 kg of mixture). Mean fodder consumption during the whole rearing period was 122.5 kg (120–125 kg) in *V. vulpes* and ca. 96.5 kg (95–98 kg) in *V. lagopus* per head (mean values from the whole rearing cycle).

Table 1. Composition of nutritive doses (%)

	Growth period ¹	Hair coat forming period ²	
	rural region	rural region	suburban region
Extruded wheat	9	13	13
Wheat bran	–	–	1
Cod fillet	5	–	–
Beef wastes	10	5	11
Porcine wastes	10	10	5
Turkey (heads and feet)	40	35	28
Poultry (heads and feet)	–	–	8
Chicken intestines	5	8	10
Fish meal	2	2	3
Blood meal	–	2	1
Meat-bone meal	3	5	4
Turkey fat	3	6	–
Beef fat	–	–	5
Water	13	14	11
Dry matter	33.28	34.67	34.28
Metabolizable energy (MJ/1kg)	6.21	7.64	7.66
Metabolizable energy (%)			
Protein	35.58	31.76	32.93
Fat	50.22	52.15	50.68
Carbohydrates	14.20	16.09	16.39

¹July, 15–September, 15

²September, 16–December, 1

The body weight of slaughtered *V. vulpes* fox was about 8 kg (7.9–8.2 kg), while in *V. lagopus* it was about 12 kg (11.8–12.1 kg). The weight of raw skin was 0.46 kg on average (0.45–0.47 kg) in *V. vulpes*, while for *V. lagopus* it was 0.50 kg (0.47–0.53 kg). The thickness of skin in the dorsal part (measured using a caliper) was similar, being 496–543 μm in *V. vulpes* and 591–650 μm in *V. lagopus*, however connective and fat (subcutaneous) tissue was thinner in *V. lagopus* (8.8 μm on average compared to 14.5 μm in *V. vulpes*).

Sample collection and preparation. The foxes were slaughtered after reaching full maturity of their fur coat. After slaughter (electric current) the skins were pulled off the animals using the bag system. After subcutaneous fat stiffening, the skins were subjected to fleshing process, i.e. removing of fat, subcutaneous membranes, and possibly muscle fibres from the surface of the skin. Cleaning of the skins from fat and other contamination was the next process, and it was conducted using sawdust in a rotating drum. Then the skins were put on fixed boards with hair turned outside and dried for a period of 48 h, and after this procedure they were removed and fur coat was combed out. They were not subjected to tanning process. Overhairs in *V. vulpes* were 56–81 mm long, and 56–74 μm thick, while these values in *V. lagopus* were 45–48 mm and 29–46 μm , respectively.

Nineteen *V. vulpes* (10 σ and 9 f) and 15 *V. lagopus* (7 σ and 8 f) individuals were selected randomly from the farm in the rural region (34 heads in total), while 18 (10 σ and 8 f) and 13 (6 σ and 7 f) (31 heads in total) animals from the farm in the urbanized region, respectively. Skin samples (with hairs), about 1 cm^2 each, were collected from dorsal and abdominal part (2 samples from each region). The hairs were accurately cut off, mixed, subjected to washing process using commercially available detergents and distilled water (rinsing), and dried at a room temperature. Hairs and skin, as well as feed mixture (homogeneous pulp) were collected to sterile plastic container for Hg content analysis. The feed mixture was collected twice, at the beginning and the end of animals rearing. Additionally, hair and skin samples were collected from one randomly chosen individual for both species and regions for scanning microscopy examinations.

Laboratory studies. The samples of hair and skin were subjected to laboratory analysis with respect to total mercury (T-Hg) content using an atomic absorption spectrometer AMA 254 (Altec

Ltd., Prague, Czech Republic). The limit of detection was 0.003 ng of Hg in the examined sample. The NIES CRM No.13 (Japan) certified reference material was used as quality control. The CRM samples were analyzed under the same manner as the experimental samples. The observed value of T-Hg in CRM samples ($n = 5$) was $4.40 \pm 0.06 \mu\text{g/g}$ (certified value of $4.42 \pm 0.2 \mu\text{g/g}$), showing the accuracy of our measurement.

In order to assess structural differences in hair and skin accounting for potential species differences in Hg accumulation, a pilot electronic microscopic study was performed. The samples of hair and skin were washed in commercially available detergent, rinsed with distilled water, and dried. Then the samples were glued on special tables using carbon strip and dusted with gold for 200 s. The samples were analyzed using a scanning electron microscope EVO LS15 (Carl Zeiss, Jena, Germany). The trichoscopic images (hair surface and cross section) were examined under $\times 100$ magnification, while the skin samples under $\times 200$ magnification.

Statistical analysis. The SAS statistical package was used for the results assessment. The calculations were performed using two-way ANOVA (GLM procedure) according to a constant model (object of research, species variety of foxes, gender). The differences between the mean Hg content in skin and hair of various fox species from two objects were determined using Duncan's Multiple Range Test. The relationship between Hg level in skin and hair was determined using straight Pearson's correlation (CORR procedure).

RESULTS AND DISCUSSION

Exposure assessment. From Table 2 it may be concluded that the feed mixture from the farm in the urbanized region contained only insignificantly higher amount of Hg (5.0 $\mu\text{g/kg}$ fresh weight or 25 $\mu\text{g/kg}$ DM on average), while in the case of the farm in the rural region this value was only by 10% lower. Generally, Hg intake with a diet was similar in foxes on both farms, however *V. vulpes* collected slightly higher amount of Hg, which may be partially explained by the fact that they lived by ca. 2 months longer than *V. lagopus*. When calculating on the final body weight, *V. vulpes* from rural and urbanized area collected about 47–48 $\mu\text{g/kg}$ (47.5 on average), while *V. lagopus* 55–60 $\mu\text{g/kg}$ (57.5 on average) of Hg. It is not

Table 2. Dietary intake of mercury (Hg) by the foxes (mean values)

Farm	Species	BW after slaughter (kg)	Hg content in feed (µg/kg)		Feed intake (kg)	Hg intake	
			I	II		total (µg)	µg/kg BW
Rural	<i>V. vulpes</i>	11.8	3.8	5.1	125	556	47.0
	<i>V. lagopus</i>	7.9	4.3	4.5	98	431	54.6
Suburban	<i>V. vulpes</i>	12.1	6.9	2.7	120	576	47.6
	<i>V. lagopus</i>	8.2	7.4	3.0	95	494	60.2

BW = body weight; I, II = subsequent fodder samplings (beginning and end of foxes rearing)

known whether these Hg amounts collected with the diet affected Hg accumulation in skin and hairs of these foxes, especially the processes of its retention and absorption in fox organism (or other *Canidae*) are not known.

The observed Hg values in fox diet were not high, the permissible content in fodders (of water content max. 12%) for animals is generally 100 µg/kg, and for dogs and cats even 400 µg/kg (Directive 2002/32/EC).

Another way for Hg intake is via respiratory tract, as the element usually occurs in gaseous form in the environment. The Hg exposure from air is difficult to assess due to lacking air Hg monitoring in Poland. It may be concluded from the Report of the state of environment in Wielkopolska in 2011 (<http://pozn.wios.gov.pl/raport-o-stanie-srodowiska-w-wielkopolsce-w-roku-2011>) that an annual average fall of particulate matter (PM 10) in the rural region was 32.9 and 28.7 µg/m³ in years 2010–2011, while it was slightly higher in the urbanized area (39.5 and 31.0 µg/m³, respectively). As the particulate matter is a mixture of various chemical elements, Hg compounds participation is difficult to estimate. The permissible average annual content of PM 10 in the EU countries is 40 µg/m³ (Directive 2008/50/EC).

Accumulation assessment. Average concentrations of T-Hg in hairs of foxes in particular groups ranged from 0.0071 to 0.0207 mg/kg (0.0139 mg/kg on average). Bocharova et al. (2013) observed differentiated Hg content in hairs of arctic foxes originating from various ecosystems, ranging from 0.57 to 10.58 mg/kg DM. The authors also noted higher Hg accumulation in adult foxes hair compared to that of juveniles, however no sex differences were revealed in this study. Many literature reports present in turn data concerning Hg accumulation in other species hair. For example, mean T-Hg concentration in unwashed hair of experimental rabbits was 0.023 mg/kg, while

maximum value was 0.053 mg/kg (Dobrzanski et al. 2007). In Canada (Quebec), in wild mink hair, mean Hg content was 30.1 µg/g, while in river otter it was 20.7 µg/g (Fortin et al. 2001). It may be concluded from another study that hair (unwashed) of wild animals in North-East India contains high Hg amounts, i.e. 74.4–168 µg/g (the highest amount in leopard cats *Felis bengalensis*). The authors relate this phenomenon with Hg content in the environment (soil, water) and feed of these predators (Dey et al. 1999).

In hair of dogs in the Upper Silesia region (Poland) maximum T-Hg concentration was 46 µg/g (26 µg/g on average) while after washing this value dropped by 38.5% (Dobrzanski et al. 2007). In turn, in urbanized areas of Korea, the content of 0.21 µg/g was noted in dog hair, and this value increased with dog age (Park et al. 2005). It was noted in Central Japan, that hair of male dogs contained 0.99 ppm and of females 0.66 ppm of Hg, while in cats it was as much as 7.40 and 7.45 ppm, which was explained by feeding fish with a considerable Hg content (Sakai et al. 1995). Also Bocharova et al. (2013) and McGrew et al. (2014) found that utilization of marine prey contributed to increased T-Hg content in arctic foxes and wolves, respectively.

In the present study, mean concentrations of T-Hg in skin (tissue) in particular groups were from 0.0033 to 0.0082 mg/kg (0.0058 mg/kg on average). Average values of 0.48–0.52 ppm (depending on tanning technique) may be found in the literature in tanned bootee leathers (Karavana et al. 2011). From another study (Aslan 2009) it may be concluded that Hg content in chromium-tanned leathers was very low, maximally 0.02 ppm (mg/kg). It is difficult to compare the present results with the above-cited covering tanned skins.

Effect of region. Figures 1A and B show that Hg concentration in hairs ($P < 0.05$) and skin ($P < 0.01$) was significantly higher in the rural environment compared to the urbanized area. In the

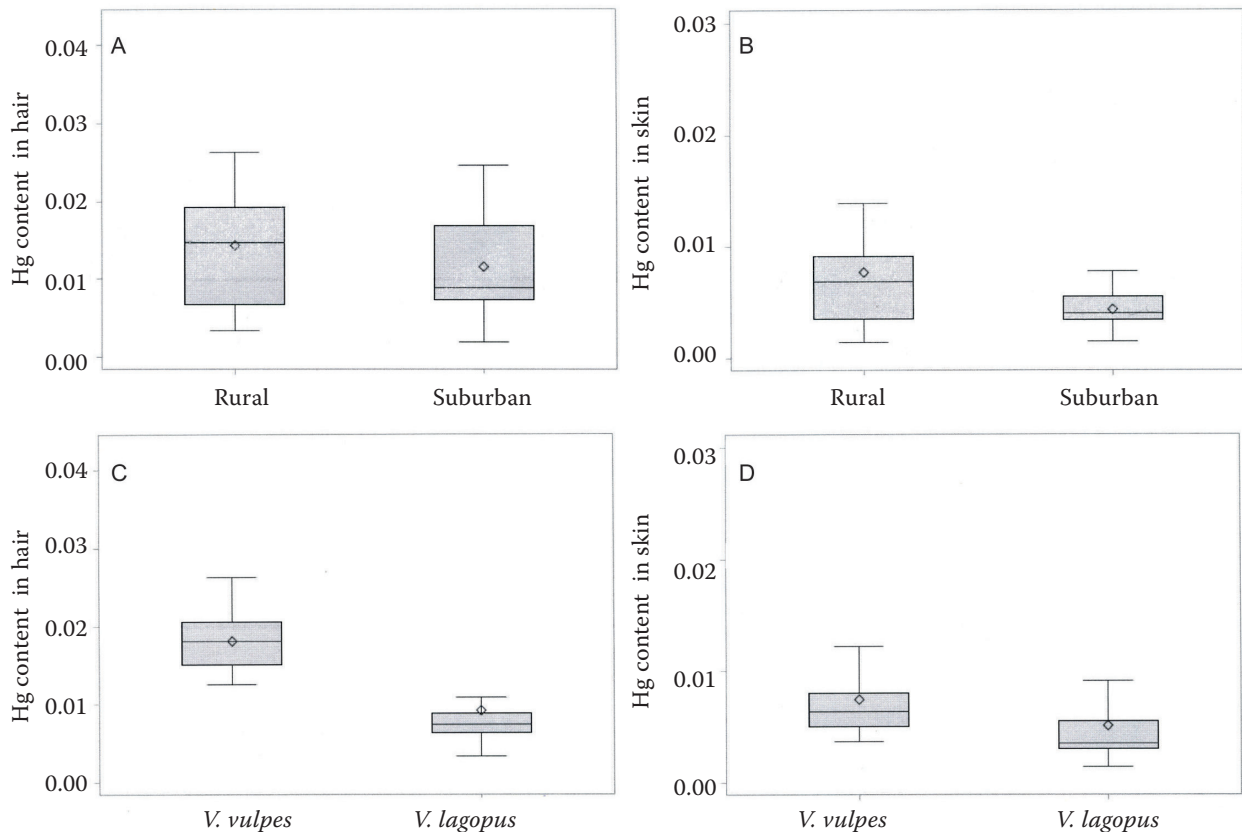


Figure 1. Mercury content in hair and skin samples of red and arctic foxes living in rural and suburban environments regional differences (A, B), species differences (C, D)

rural area, mean Hg content in hairs was twice higher compared to skin. In the urbanized region, this difference was even higher, nearly three-fold. The higher Hg content in the coats of the foxes farmed in the rural region is somewhat surprising. This points to the influence of the environment, with similar dietary Hg intake. The inspection of areas around both farms revealed no burdensome industry near the farm in the urbanized region, while natural gas mining plant and busy communication road were localized in the rural region. Presumably, these environmental factors crucially affected air pollution and an increased Hg accumulation in the rural area farmed foxes. It may be concluded from the literature data, that organs and muscles of wild living foxes (*Vulpes vulpes*) in suburban area contained twice higher amount of Hg compared to those from rural region in Croatia (Bilandzic et al. 2010). Also Szkoda et al. (2012) noted the highest Hg accumulation in the game in the industrialized region of Upper Silesia in Poland.

Effect of species. Figures 1C and D demonstrate that significantly ($P < 0.01$) higher Hg amounts were accumulated in hairs and skin of silver foxes (*V. vulpes*) compared to arctic ones (*V. lagopus*). This probably resulted from longer exposure of silver foxes to contamination, since they lived by two months longer than arctic foxes. This was also demonstrated in the study of Filistowicz et al. (2012) who observed significantly higher accumulation of Cr and Cu in hairs, and Cr, Ni, and Zn in skin of *V. vulpes* compared to *V. lagopus*. In turn, Kalisinska et al. (2012) revealed very different values of Hg content in liver and kidneys of *V. vulpes* (0.04–0.52 (0.14 on average) in liver, and 0.09–0.84 (0.30 on average) mg/kg DM in kidneys).

During our electron microscopic pilot study, the two fox species showed similarities in hair internal structure, while a less intense “mosaic” of the external layer of hair was noted in *V. vulpes*. Moreover, the skin of *V. lagopus* was more compact. Although we cannot draw any conclusion on these findings due to the low sample size, further studies

Table 3. Mean values and standard deviations (in parentheses) of mercury content in hairs and skin of foxes depending on farm, species, and gender

Farm	Species	Gender	Hairs	Skin
Rural ($n = 34$)	<i>V. vulpes</i> ($n = 19$)	♂	0.0190 ^{ABCa} (0.0053)	0.0078 ^{Aa} (0.0026)
		♀	0.0207 ^{DEFab} (0.0040)	0.0074 ^{bcd} (0.0028)
	<i>V. lagopus</i> ($n = 15$)	♂	0.0074 ^{ADG} (0.0037)	0.0038 ^{Ba} (0.0022)
		♀	0.0149 ^{bcd} (0.0120)	0.0082 ^{BCDb} (0.0060)
Suburban ($n = 31$)	<i>V. vulpes</i> ($n = 18$)	♂	0.0153 ^{GHief} (0.0073)	0.0052 (0.0013)
		♀	0.0177 ^{fg} (0.0027)	0.0059 (0.0016)
	<i>V. lagopus</i> ($n = 13$)	♂	0.0090 ^{BEHceg} (0.0031)	0.0036 ^{Cc} (0.0008)
		♀	0.0071 ^{CFId} (0.0007)	0.0033 ^{Add} (0.0009)

^{A–I} means in columns marked with the same upper case differ significantly at $P \leq 0.01$

^{a–g} means in columns marked with the same lower case differ significantly at $P \leq 0.05$

are needed in this direction as the observed differences may explain the accumulation differences.

Effect of gender. Table 3 shows that females had accumulated higher amount of Hg in hair for most of the time, except in the case of the urbanized *V. lagopus*, where higher Hg values were found in males. In skin, similar Hg content was observed in males and females, only in *V. lagopus* farmed in the rural area an increased Hg content was noted in females. It is difficult to explain, especially because the animals were given similar fodder and were housed under similar environmental conditions. Some genetic and metabolic Hg availability dispositions in these animals cannot be excluded. The study conducted by Sakai et al. (1995) confirms this hypothesis, because these authors noted a significantly higher Hg concentration in hair of male dogs compared to females, while no gender effect was observed in cats. Also Hazelhoff et al.

(2012) demonstrated gender influence on Hg accumulation in rat kidneys.

Correlation. Table 4 points to a significant relationship between Hg content in hairs and skin observed in the group of *V. vulpes* males ($r = 0.764$) and *V. lagopus* females ($r = 0.950$) from the rural region. Significant relationships were also observed within *V. lagopus* species on the farm localized in rural region ($r = 0.919$) and in all animals on both farms ($r = 0.796$). These high correlation coefficients unequivocally point to physiological and metabolic relationship between raw skin and its product, i.e. the hair. Filistowicz et al. (2011, 2012) noted a significant relationship between skin and hair content by chromium ($r = 0.559$), zinc ($r = 0.476$), and copper ($r = 0.621$) in *V. lagopus* and *V. vulpes*. It is worth to note, that Hac et al. (1996) found positive correlation between Hg content of the hair, kidneys, blood, and urine of

Table 4. Straight correlations (Pearson's) between mercury content in hairs and skin of foxes depending on farm, species, and gender

Farm	Species	Gender	r	
Rural ($n = 34$)	<i>V. vulpes</i> ($n = 19$)	♂ ($n=10$)	0.764*	0.449
		♀ ($n = 9$)	0.235	
	<i>V. lagopus</i> ($n = 15$)	♂ ($n = 7$)	0.528	0.919**
		♀ ($n = 8$)	0.950**	
				0.796**
Suburban ($n = 31$)	<i>V. vulpes</i> ($n = 18$)	♂ ($n = 10$)	0.342	0.419
		♀ ($n = 8$)	0.711	
	<i>V. lagopus</i> ($n = 13$)	♂ ($n = 6$)	0.393	0.363
		♀ ($n = 7$)	0.354	

*significant at $P \leq 0.05$, **significant at $P \leq 0.001$

rats intoxicated with mercury chloride. Statistically significant correlation ($r = 0.321$) was noted between Hg concentration in the hair and liver content of golden jackal (*Canis aureus*) (Malvandi et al. 2010). Thus, the noninvasively collected hair samples may be useful in the assessment of heavy metals bioaccumulation in animal organs.

CONCLUSION

Although fed a similar fodder, the present study proved that Hg concentrations accumulated in skin and hair samples from arctic and silver foxes reared in rural and urbanized environments were different. Rural foxes accumulated the highest Hg amounts, which may reflect the pollution of the air due to an industrial plant and a highway close to the farm. Hair of female silver foxes showed the highest Hg content, however the mechanisms behind the species and gender differences in Hg accumulation need to be addressed in further studies. A significant correlation between hair and skin Hg content found in the present study reinforces previous findings that non-invasively collected hair samples analyses are a good tool for assessing the heavy metal load of different environments.

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