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Mercury Content in Organs and Tissues of Indigenous (*Vulpes vulpes* L.) and Invasive (*Nyctereutes procyonoides* Gray.) Species of Canids from Areas Near Cherepovets (North-Western Industrial Region, Russia)

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Abstract Trophic and spatial components of ecological niches of two canids native *Vulpes vulpes* and introduced *Nyctereutes procyonoides* are overlapping partially in the studied region. Maximum concentrations of mercury in predatory mammals of Canidae family from surroundings of Cherepovets have been determined in liver and kidneys (over 0.50 mg/kg wet weight), with minimal concentrations in brain (<0.2 mg/kg wet weight). The amount of mercury in the same organs of the red fox and raccoon dog is not significantly different. These levels of mercury content are noticeably higher than those in the predators of Canidae family that inhabit territories of Europe lacking the local sources of mercury. At the same time, absolute values of metal quantity are commensurable with the levels registered in predators from the mercury polluted regions of Spain and Poland.

Keywords Mercury · Mammalia · Carnivora · Canidae · Common fox · Raccoon dog

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Mercury (Hg) is able to accumulate effectively in the food chains, causing a wide spectrum of negative impacts on the mammals, above all, affecting their central nervous system (Heinz 1996; Wolfe et al. 1998; Driscoll et al. 2007). The total amount of anthropogenic metal mercury emission at present time in Russia is about 4 % of global releases (Wilson et al. 2006; UNEP 2008). The main anthropogenic sources of mercury are metallurgy, chlorine-alkaline industry, combustion of natural organic fuel and solid domestic waste (UNEP 2008).

In Cherepovets, an industrialized region in the north-western portion of European Russia, has a large metallurgic processing complex that has operated since 1956. This complex is the second largest in Russia, smelting more than 20 millions of tons of cast iron and steel per year and producing the finished rolls of ferrous metals. Cherepovets is the regional center for other types of industry which could also contribute to local mercury emission to the environment. Evidence of increased mercury emission from Cherepovets to the regional ecosystem comes from elevated concentrations (1.0–3.0 mg Hg/kg ww) repeatedly detected in the muscle tissues of perch from isolated lakes situated within a radius of 100 km from Cherepovets (Haines et al. 1992; Stepanova and Komov 1997).

More intense migration and accumulation of toxic organometallic compounds occurs in aquatic food-webs (Wiener et al. 2002). Therefore, the investigation of predatory mammals subject to mercury accumulation in aquatic ecosystems has largely been carried out on the otters and minks (Scheuhammer et al. 2007). The characteristic properties of uptake and distribution of mercury in terrestrial predatory mammals (omnivorous and scavenger species) have received much less attention compared to these semiaquatic predatory mammals, particularly in Russia (Heinz 1996; Shore et al. 2001). This is true in spite

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of the fact that over the last decade investigations demonstrate that the data on the mercury content in organs and tissues of predators from the Canidae family offer an important estimation of overall mercury contamination in these ecosystems (Munthe et al. 2007). The most abundant wild canid species in the studied region are the native red fox and introduced raccoon dog (Priroda Vologodskoi oblasti 2007). Hence, the objective of this work was to study the mercury content in organs of predatory mammals from the Canidae family, which inhabit various ecosystems surrounding the largest industrial complex in the North-Western portion of Russia.

Materials and Methods

The samples of Canidae tissues and organs were collected in 2007–2011 in the surroundings (within a 30 km radius) of Cherepovets (59°04′25″–59°10′53″N, 37°39′37″–38°00′40″E), Vologda district, Russia (Fig. 1).

The material gathered consisted of 96 samples from 21 individuals from 2 species from the Canidae family: raccoon dog (*Nyctereutes procyonoides* Graj) (n = 14, 9 males and 5 females) and red fox (*Vulpes vulpes* L) (n = 6, 4 males and two females). All studied individuals were sexually mature.

Raccoon dog introduction in the European North has begun in 1934 with maximum abundance of this species registered in late 1950's (Danilov 2009). Despite the fact that introduced species—raccoon dog possesses an ecological niche similar to that of the indigenous species—red fox; competition between these two species is weak. This may be due to the peculiarity of raccoon dog biology—hibernation, which lasts from November–December to March–April in the studied region. During this period, when food is scarce for predators, native species does not suffer competitive pressure from the introduced species (Danilov 2009).

The samples were taken from local, licensed hunters belonging to the regional hunting organizations and associations. Due to the differential carcass integrity of the samples, particular those of red foxes and raccoon dogs captured in the south-western portion of the collection territory not all organs could have been studied (Site No. 2). After sex determination, samples of different organs and tissues (liver, kidneys, muscles, brain) were put into plastic sachets, frozen and kept at −16°C. Previous studies have shown that water content in muscles and liver of predatory mammals is 70 % and around 80 % in the brain and kidneys (Lanocha et al. 2014). We did not perform evaluations in dried tissues in the present study that is why values of mercury concentration given in brackets are

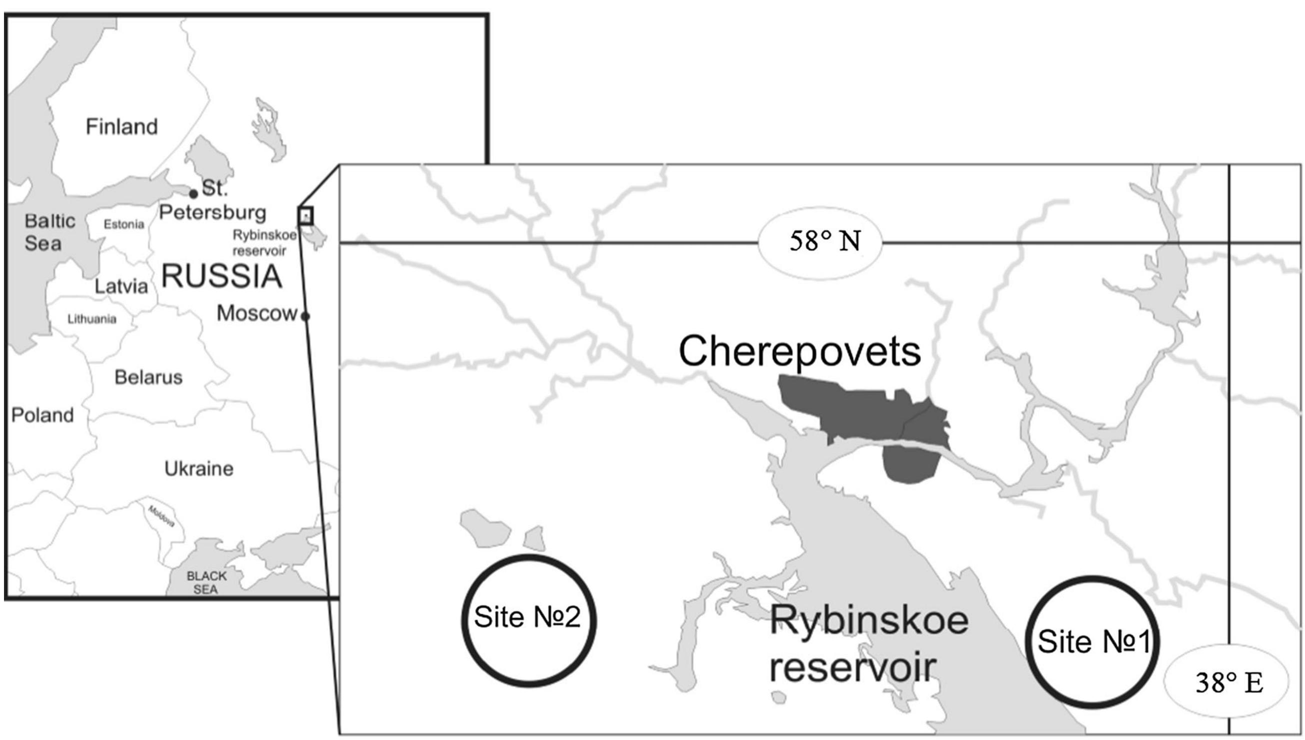

Fig. 1 Sampling sites

values of wet weight recalculated into dry weight (dw). Mercury content in samples was determined using a mercury analyzer (PA-915 + with PYRO device, Lumex®), without preliminary preparation of the samples at IBIW RAS (direct burning). The accuracy of the analytical methods was evaluated using certified biological materials DORM-2 and DOLM-2 (Institute of Environmental Chemistry, Ottawa, Canada). The method's limit of detection (LOD) 0.001–5.00 mg/kg.

The mercury content in tissues and organs of mammals has been reported as wet weight (mg/kg ww) and presented as average values and their mean errors (x ± SE). The results have been processed statistically using the analysis of variance method (ANOVA), noting differences at the significance level $p \leq 0.05$ (Sokal and Rohlf 1995). All data were checked for normality using a Shapiro-Wilks test. All mercury measurements were found be non-normally distributed. Spearman's (r_S $p < 0.05$) was used to examine the correlation between organs mercury concentrations.

Results and Discussion

The mercury concentrations in the organs and tissues of the investigated species of canines differed by two orders of magnitude, varying from 0.01 to 0.96 mg Hg/kg ww (Table 1). The average concentration values of mercury in the organs and tissues of fox decreased in the order: liver > kidney > muscle > brain. Minimal variability of mercury content was detected in liver and kidney tissues, with a range of approximately twofold to sixfold from minimum to maximum values, while maximum variability was observed in fox brain tissues (80 times). Mercury concentrations in liver and kidneys of animals did not differ statistically amongst themselves, but they were significantly greater than concentrations of mercury measured in brain samples. Intermediate values of mercury were detected in muscle and they did not differ significantly from the content in brain (the organ with the least mercury content) or that of the liver and kidney (organs with maximum metal content).

Discrepancy in the number of samples of different organs (n) is due to different degree of preservation of animal carcasses obtained from hunters, in some cases it was impossible to collect all organs needed for analysis.

The mercury amount in the similar organs of raccoon dog and fox did not differ statistically (Table 1). The average values of mercury content in organs and tissues of raccoon dog decreased in the order: liver > kidneys > muscles > brain.

The difference between minimal and maximal mercury concentrations in liver, kidneys, muscles and brain were 8 to 18-fold (Table 1).

The mercury content in liver is significantly greater than in kidneys, while kidney content was significantly greater than that observed in muscle of the raccoon dog.

Absolute values of mercury concentrations in liver, kidneys and brain of animals (n = 10) collected along the eastern bank of the Rybinsk Reservoir (Site No. 1) exceeded by twofold the concentration values in animals (n = 3) collected along the South-Western portion of the region (Site No. 2) (Figs. 1, 2). At the same time, no significant differences in metal content in muscles and brain were revealed.

A significant positive correlation between mercury content in the liver and other organs of the raccoon dog ($r_S = 0.55$–0.83, $p < 0.01$, n = 8–13) and organ pairs muscles-brain ($r_S = 0.96$, $p < 0.1$, n = 14) was found.

Significant correlation between mercury content in the brain and muscles of fox ($r_S = 0.98$, $p < 0.01$, n = 6) was observed as well. There is a possible dependency between mercury content in other organ pairs but the sample are too small to perform any statistical analysis (n = 2–3). No significant differences in metal content in organs and tissues from males and females of the investigated species were detected. Mercury content in the liver was 0.57 and 0.53 mg/kg, 0.24 and 0.27 in kidneys, 0.02 and 0.02 in the brain, 0.08 and 0.05 in muscles of both males and females of raccoon dog.

Average values of total mercury in liver and kidney tissues of predatory mammals (Canidae family) collected from areas surrounding Cherepovets (0.25–0.50 mg Hg/kg of ww or 1.25–1.66 mg Hg/kg dw) were an order of magnitude greater than metal concentrations in respective organs of

Table 1 Mercury content [mg/kg ww and mean errors (x ± mx)] in organs and tissues of raccoon dog (*Nyctereutes procyonoides*) and red fox (*Vulpes vulpes*), [minimum and maximum index values—under the line (n)]

	Hg, mg/kg ww			
	Muscles	Liver	Kidneys	Brain
Red fox	0.09 ± 0.07^{ab}	0.31 ± 0.16^{b}	0.28 ± 0.04^{b}	0.03 ± 0.01^{a}
	0.01–0.32 (6)	0.11–0.64 (3)	0.18–0.42 (3)	0.01–0.08 (6)
Raccoon dog	0.10 ± 0.035^{a}	0.50 ± 0.084^{c}	0.25 ± 0.07^{b}	0.03 ± 0.01^{a}
	0.03–0.54 (14)	0.07–0.96 (14)	0.07–0.60 (14)	0.01–0.18 (14)

[a,b,c] Values with different letter superscript differ significantly in organs within species (in lines) with significance level $p \leq 0.05$ (ANOVA-test)

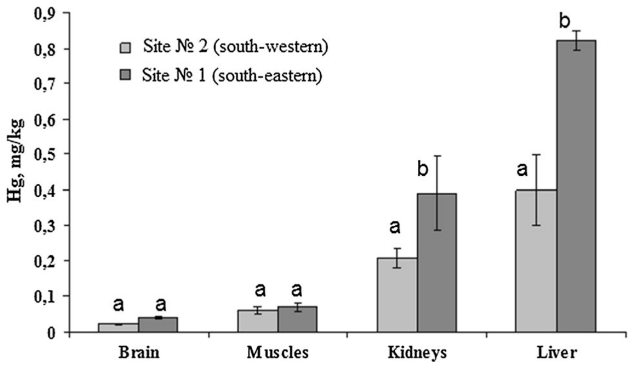

Fig. 2 Mean mercury content (mg/kg ww) measured in organs and tissues of raccoon dog from the two sites of investigated region and mean errors (x ± mx).*a*, *b* values with different letter differ significantly in organs within species with significance level $p \leq 0.05$ (ANOVA-test)

wild foxes bagged from 2004 to 2009 in Western parts of Poland, Croatia and Italy (0.007–0.06 mg Hg/kg ww or 0.02–0.30 mg Hg/kg dw) (Kalisinska et al. 2009; Bilandzic et al. 2010; Alleva et al. 2006). The data obtained in this investigation are comparable with earlier metal concentrations detected in polar fox (0.20–0.80 mg Hg/kg ww or 0.66–2.66 mg Hg/kg dw) captured in Canada in 1972–1990 (Champoux et al. 1999). At the same time, mercury concentrations in liver and kidney of animals from the area surrounding Cherepovets were similar to values in respective organs of red fox (0.30–1.28 mg Hg/kg of ww or 1–6.40 mg Hg/kg of dw), caught in mercury polluted regions of Spain and on the island of Milin (Poland) (Millan et al. 2008; Kalisinska et al. 2009).

Mercury concentration in liver and kidneys of canines reared in captivity on farms does not exceed 0.05 mg Hg/kg of ww (0.16 mg Hg/kg dw) and has been considered by some authors to be an index of metal geochemical background (Farrar et al. 1994; Cybulski et al. 2009). In the organs of specimen investigated in this study the average mercury content was 4–7 times greater in kidneys and 6–15 times greater in liver than this reference index concentration. That is why mercury concentrations detected in the organs and tissues of these predatory mammals during our investigation provide evidence of an increased metal content in the terrestrial ecosystem of the Cherepovets region.

The total mercury concentrations in organs and tissues of the predatory mammals of Canidae family from the surrounding areas of Cherepovets registered during this research are, on average, lower than the indices detected in representative organs from mustelids (otters and minks) in most other studies. The metal content in liver of otters and minks from North America varied between 0.26 and 8.66 mg Hg/kg ww (0.86–28.66 mg Hg/kg dw) and 0.85–10.0 mg Hg/kg ww (2.80– 33.3 mg Hg/kg dw), in brain 0.06–10.2 mg Hg/kg ww (0.30–51 mg Hg/kg dw) (Evans et al. 2000). High mercury concentrations in organs of mustelids are likely due to their preferred diet of fish (Wiener et al. 2002). Hydrobionts are rarely present in canids' diet. The terrestrial diets of Canidae species are generally lower in mercury content as compared to fish, particularly in mercury contaminated ecosystems (Danilov 2009).

As it has been shown above, the increased mercury content in organs of mammals can be connected with the peculiarities of dietary preferences: maximum values have been determined in the animals which feed mainly on fish and other hydrocoles (Wiener et al. 2002). The surroundings of Cherepovets, where the investigated specimen of raccoon dogs have been caught, consists of diverse biotopes and are equally distant from the industrial centre. The animals caught in the southeastern part of the investigated region most probably prey on hydrobionts, and also birds and mammals trophically-linked with the aquatic ecosystems (the considerable part of this territory is situated on the bank of the Rybinsk Reservoir). There are no large reservoirs on the territory of southwestern part of the investigated region, and it is situated farther away from the Rybinsk Reservoir. Thus, hydrobionts were not likely as abundant in the diet of raccoon dogs from that site. In addition to that, atmospheric transfer occurs mainly from the west eastwards likely causing lower mercury content in tissues of animals from the south-western site (Site No. 2) as compared with south-eastern site (Site No. 1). Earlier it was shown that mercury accumulation in tissues of marten from the east part of the region was significantly lower (liver—0.22; kidney—0.38 mg Hg/kg ww or liver—0.73 and kidney—1.9 mg Hg/kg dw) when compared with such from the western part (liver—0.45; kidney—0.84 mg Hg/kg ww or liver—1.50 and kidney—4.20 mg Hg/kg dw) where the metallurgical plant is situated (Komov et al. 2012).

In the majority of the research carried out previously, the mercury content has been determined in liver and kidney of mammals, which are the organs that have the function of contaminant detoxification (Cristol et al. 2008). The high levels of metals in liver of animals can be used as an index of environmental contamination, while the specific metal concentration in the organ reflects the degree of the long-term influence of mercury on the ecosystem. However, to estimate the influence of mercury in mammals, the concentration of mercury in the liver and kidneys alone is not sufficient, as the significant portion of mercury in these tissues is often present in a less toxic, inorganic form. Additionally, the proportion of methylmercury is smaller in liver and kidneys, as compared to concentrations found in brain and muscle tissues (Strom 2008).

As in the animals from the surroundings of Cherepovets, the amount of mercury has been determined in not only liver and kidneys, but also in brain and muscles. This can provide complementary information for the estimation of the status of their population and for the definition of mercury distribution within an organism. The neurotoxic action of metal manifests itself initially by injury to the organs of the central nervous system (Heinz 1996; Wolfe et al. 1998; Driscoll et al. 2007). Moreover, the dependence of metal accumulation in the brains of otters on factors such as local geological characteristics, chemical composition of water, sex and age of animals has been recorded. For the other organs this correlation has not been determined (Strom 2008). The muscular tissue makes up a significant portion of weight of mammals body and apparently serve as the main repository for accumulated mercury in an organism.

Correlations of metal distribution between the organs in canines from the surroundings of Cherepovets coincide with the similar data for the canines grown on the farms and for the otters of North America (Cybulski et al. 2009; Strom 2008).

In the present research it was determined that the metal quantity in brain of mammals was 8–10 times less than in the liver, that is due to the different morphology of each tissue of the investigated organs. Additionally some physiological processes can limit the metal penetration into the central nervous system. Previously, in studies of small mammals, the supposition has been made that the accumulation and distribution of mercury between various tissues and organs in organism depends on the biochemical composition, metabolism and functionality of organ (Ulfvarson 1970). Similarly, in the investigations carried out in North America on otters it has been observed that the quantitative ratio of accumulated organic and non-organic forms of mercury are different among internal organs (Strom 2008).

Statistically significant correlations between mercury concentrations in organs and tissues (muscles—liver, liver—kidneys, liver—brain in raccoon dogs and muscles—brain in common fox caught in the surroundings of Cherepovets) were comparable to the earlier estimated correlations for the organs [liver—muscles ($r_S = 0.74$, $p < 0.01$), kidneys–liver ($r_S = 0.60$, $p < 0.01$), kidneys—muscles ($r_S = 0.57$, $p < 0.01$)] in the fox from Poland (Kalisinska and Palczewska-Komsa 2011). We did not have opportunity to get all the necessary organs for the analysis of accumulation and distribution of mercury. However, based on the elevated concentrations of mercury observed in liver and kidneys, as well as the significant correlations between the tissue concentrations, it is possible to suppose that an elevated mercury content is likely in the organs not evaluated in this study.

The difference in mercury content of organs and tissues from male and female raccoon dogs sampled in our study of canines from the surroundings of Cherepovets was not significant, which is in accordance with the earlier data obtained from other predatory mammals of Canada and USA (Fortin et al. 2001; Yates et al. 2005). At the same time O'Connor and Nielsen (1981) have noted that the mercury content in organs of male otters was greater than that found in the same organs of female otters.

Sublethal concentrations of mercury have been estimated to be approximately one-third that of the average lethal concentration in various organs of mink and otter, at about 3.0–10.0 mg/kg ww (Halbrook et al. 1994). In the laboratory, it has been observed that a mercury content exceeding 2.8–3.3 mg/kg ww in the liver of dog was lethal (Farrar et al. 1994). Hence, it is not inconceivable that the representatives of Canidae family are more sensitive to the mercury poisoning in comparison with the terrestrial piscivores. However, in a number of studies there is a supposition that mercury accumulation in organs and tissues of predatory animals can adversely affect survival in the wild as a consequence of reduced visual acuity and a subsequent reduction in the ability to hunt, resulting in starvation and decreased reproduction (Wolfe et al. 1998). No extremely toxic, presumed lethal concentrations of mercury were observed in the organs and tissues of animals from this investigation of Canidae that inhabit the surroundings of the large industrial complex of Cherepovets in North-West Russia. However, it is impossible to understand the true nature of the risk of mercury to these mammals due to a lack of information on toxicity thresholds for the species sampled. The elevated mercury concentrations registered in single individuals demonstrate a real potential for functional abnormalities to occur based on mercury content. Thus, continued efforts to monitor concentrations of metals in tissues and organs of the representatives of Canines are recommended for this region.

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