

RED FOX, *VULPES VULPES* L., AS A BIOINDICATOR OF ENVIRONMENTAL POLLUTION IN THE COUNTRYSIDE OF CZECH REPUBLIC

Jana Sedláková¹, Petr Řezáč¹, Vladimír Fišer², Josef Hedbávný³

¹Department of Morphology, Physiology and Animal Genetics, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

²Department of Information Technology, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

³Department of Chemistry and Biochemistry, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

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Abstract

The objective of this study is to determine the accumulation of heavy metals in the tissues of the small intestines of red foxes from 4 different areas of the northern part of the Czech Republic. The red foxes were relocated from countryside and free nature to the neighborhood of big cities in the last two decades, so it could be great indicator of pollution in these four different areas, with the focus on countryside problems with pollution of heavy metals. The heavy metal (Cd, Pb, Cr, Cu, Zn, Mn, Ni) concentrations in 27 samples and the relationships between heavy metal concentration in red fox tissue and the location of sample were investigated. The highest concentrations of Pb (0.684 mg.kg⁻¹), Cr (0.162 mg.kg⁻¹), Ni (0.235 mg.kg⁻¹) and Zn (19.76 mg.kg⁻¹) were found in tissues from mountain areas. The highest concentrations of Cd (0.047 mg.kg⁻¹), Cu (1.474 mg.kg⁻¹) and Mn (2.025 mg.kg⁻¹) were measured in tissues from industrial agglomeration around the city Hradec Králové. Pb and Cr concentrations in tissues of red foxes from two areas (environs of the city Krasna Lipa and Hradec Kralove) exceeded the permissible food consumption limits.

Keywords: heavy metals, cadmium, lead, chrome, copper, zinc, manganese, nickel countryside pollution

INTRODUCTION

Air in populated areas is often contaminated with pollutants produced by human. The greatest problems nowadays are suspended particles and substances bound to them, such as polycyclic aromatic hydrocarbons and heavy metals. Heavy

metals in the air pose a significant potential threat to the environment. It is commonly known that environmental pollution significantly influences the bioaccumulation of chemical elements in animals and plants (Rogowska *et al.*, 2009; Mariet *et al.*, 2010). Wild animals are naturally exposed to heavy metals in their natural habitats.

Field studies have shown that high levels of heavy metals in the environment can be monitored in the internal organs of free-living mammals (Piskorova *et al.*, 2003). Determination of heavy metals is important in carnivorous mammals because they are at the top of the food chain, and therefore they may be very sensitive to any ongoing biomagnification processes (Bilandžić *et al.*, 2010).

The red fox (*Vulpes vulpes*) heavily populates the northern hemisphere and is the most abundant wild carnivore living in the Czech Republic. The red fox is an omnivore animal with a varied diet and easily adapts to environmental conditions. Red foxes settle small territories and often occurs in urbanized areas (Tsukada, 1997), and therefore may serve as a surrogate species for the assessment of toxic health hazards.

The aim of this study was to evaluate the level of environmental contamination in the countryside of northern part of the Czech Republic by monitoring heavy metals in tissues in the wall of the small intestine of red foxes (*Vulpes vulpes*).

MATERIALS AND METHODS

Samples of intestine tissues of 27 red foxes were collected largely in the northern part of the Czech Republic, neighboring Germany and Poland, from 2009 to 2010. After evisceration of about 100–300 g of intestines they were separated, put into polyethylene bags and frozen at -18°C immediately upon returning from the hunts for further processing. For the determination of heavy metals in frozen samples, the atomic absorption spectrometry method was used (Dědina, 1987). Samples were divided into 4 groups based on the area of catch. Boundaries of each area were defined using the landscape relief and taking potential close and distant sources of pollution into account (Fig. 1).

Areas of sample collection

Area I – environs of the city Krasná Lúpa, northern part of Czech Republic ($50^{\circ}46' - 51^{\circ}1' \text{ N}$, $14^{\circ}17' - 14^{\circ}38' \text{ E}$).

Main feature of the landscape: the transition of the Lusatian Mountain ridges into the landscape of sandstone rock towns. In the defined area, three large protected areas meet: Czech Switzerland, Elbe Sandstones and the Lusatian Mountains. In the past, the highest parts of mountains were damaged by air pollution mostly from the Polish power plants Turów and German power plants Hirschfelde, Hagenwerde and Boxberg, which are located within 50 km.

Area II – around the city Frýdlant, northern part of Czech Republic ($50^{\circ}55' - 51^{\circ}0' \text{ N}$, $15^{\circ}1' - 15^{\circ}10' \text{ E}$).

The area is separated from the rest of the state by the massif of the Jizera Mountains. From the point of view of landscape, the alluvial plain of the river Smědá is precious to the community, with its meanders, oxbows and gravel sediments and with the plants and animals of wetlands, river floodplains and hillside forest. The area is polluted by loess of brown coal mines in Polish Bogatynia.

Area III – under the Jested Mountain, northern part of Czech Republic ($50^{\circ}38' - 50^{\circ}49' \text{ N}$, $14^{\circ}47' - 15^{\circ}8' \text{ E}$).

The majority of this area is situated on the slope of the Jested ridge, south of the town Liberec. The ridge extends for nearly 60 kilometers from northwest to southeast. The landscape is affected by its historical use for agriculture and forestry. There are pastures on the slopes of the ridge, used for grazing sheep or cattle, kept for the maintenance of the land. The most significant and particularly large sources of air pollution remain the heating plant and municipal waste incinerator in Liberec.

Area IV – environs of the city Hradec Králové, eastern part of the Czech Republic ($50^{\circ}10' - 50^{\circ}26' \text{ N}$, $15^{\circ}39' - 16^{\circ}12' \text{ E}$).

In the plains, near the rivers Elbe and Eagle, one can find numerous oxbows, lakes as remnants of the extraction of sand, and large pine forests. Sources of air pollution in the area are numerous industrial companies in the city. One mustn't forget the long-range transport of pollutants from sources outside the city. Within 50 km distance there are the industrial companies Synthesia Pardubice – Semtín, Opatovice on Elbe and the Chvaletice power plant.

Element determination

To determine the elements Pb, Cd, Ni and Cr atomic absorption spectrometry (AAS) (GBC Australia) was used, with an electrothermal atomization variant (ETA AAS). For Cu, Mn and Zn flame version of atomization (FAAS) was used with a flame $\text{C}_2\text{H}_2/\text{air}$. An AAS AA30 spectrometer with graphite furnace GTA 96 by Varian Australia Company was used for measurements. To suppress the background for Pb and Cd correction by D2 a lamp and matrix modifier ($\text{Mg}(\text{NO}_3)_2 + \text{NH}_4\text{H}_2\text{PO}_4$) was used. For Cr ascorbic acid was used as matrix modifier. Mineralized samples were prepared at 210°C by a microwave solvent extraction labstation ETHOS SEL by Milestone, Italy. Concentrated

HNO₃, distilled in BERGHOF Company equipment, was used as a solvent. Tissue sample weight was in the range from 0.16 to 0.6 g and the volume of mineralization reagents was 5 ml HNO₃ + 5 ml H₂O (deionized distilled water). After cooling, samples were filtered into 25 ml volumetric flasks. Element concentrations were measured in mg. l⁻¹ and converted to mg.kg⁻¹ in fresh material.

Statistical analysis

The results obtained were processed by the statistical program *Statistika ver.10* from the StatSoft Company and *Adstat ver.1.25* from TriloByte. At first, we focused on the analysis of one-dimensional data. Exploratory data analysis indicated an asymmetrical distribution of the sample data. Therefore, we expected incorrect values of classical and robust estimation of parameters. Consequently, the Box-Cox transformation method was used.

In the case of small samples ($4 < n < 20$) use of the Horn procedure of pivot measures is recommended (Meloun *et al.* 2001). Interval estimate gives a range of possible values with a pre-chosen probability. Lower limit $L_L < \mu < \text{upper limit } L_U$. For significance level $\alpha = 0.05$ the 95 % confidence interval of the measure of location was calculated. To assess the significance of variability sources in the data, ANOVA (Analysis of Variance) was used. For comparing treatment group means, after the ANOVA null hypothesis of equal means was rejected, Tukey's post-hoc test was used.

RESULTS AND DISCUSSION

The highest values of concentrations of Pb (0.686 mg.kg⁻¹), Cr (0.162 mg.kg⁻¹) and Ni (0.235

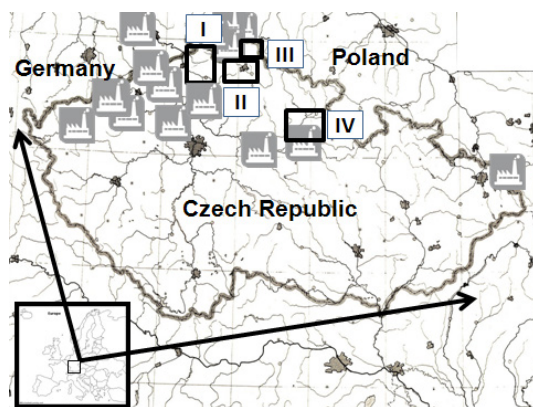
mg.kg⁻¹) were measured in Area I, the highest concentration of Zn was measured in Area III. The highest concentrations of Cd (0.047 mg.kg⁻¹), Cu (1.474 mg.kg⁻¹) and Mn (2.025 mg.kg⁻¹) were measured in Area IV (Tab. I, Fig. 2).

Significantly higher levels of Cd were found in Area IV (0.048 mg.kg⁻¹). Significantly higher levels of Pb were found in the Areas I and IV. Sample analysis detected a statistically significant relationship between the content of Cd ($P = 0.018$) and Pb ($P = 0.010$) in the intestine of foxes and areas. Pb significantly hampers the environment in areas I and IV.

Relation between content of Cr, Cu, Zn, Mn and Ni and the region was statistically inconclusive. Environmental contamination in observed area was evaluated by comparing the content of heavy metal in fox tissues with set in Regulation No. 298/1997 of the Ministry of Health.

The results of this study indicate that Pb content in the tissues of animals in areas I and IV can exceed hygienic limits for food (Pb 0.5 mg.kg⁻¹). The detected upper limit of the 95 % confidence interval of Cr content in fox intestines from areas I and IV was higher than allowed by legislation (Cr 0.2 mg.kg⁻¹). No values of heavy metals in samples from area II exceeded hygienic limits for food. That is surprising considering the fact that the Polish power plant Turow lies nearby.

There is a general presumption that increased concentration of heavy metals can be expected in urban, suburban and industrial emission-affected areas. Similar conclusions were drawn by Bukovjan (1997) in central Bohemia (Czech Republic), Bilandžić *et al.* (2010) near Zagreb (Croatia), Piskorova *et al.* (2003) in the Central region Zemplin of the Slovak Republic, Dip *et al.* (2001) around Zürich (Switzerland) and Jankovská *et al.*



1: Study areas,  – the biggest coal power stations in the Czech Republic and neighboring countries, operating during the year 1992

I: Areas, numbers of viscerated foxes, re-transformed mean and 95 % confidence interval of heavy metal concentration in intestine tissues of red foxes.

Area		Metal (mg.kg ⁻¹)						
		Cd	Pb	Cr	Cu	Zn	Mg	Ni
I n=7	$\bar{x}_R$ $\bar{x}$	0.028 ^b	0.684 ^b	0.162	1.115	19.4	1.94	0.235
	L _L	0.008	ND	ND	0.675	13.03	1.124	0.084
	L _U	0.052	1.524	0.542	1.649	30.29	2.684	0.304
II n=6	$\bar{x}_R$ $\bar{x}$	0.003 ^a	0.190 ^a	0.039	1.098	18.96	1.227	0.174
	L _L	ND	0.048	ND	0.870	16.05	0.241	0.086
	L _U	0.040	0.377	0.123	1.370	27.21	2.550	0.294
III n=8	$\bar{x}_R$ $\bar{x}$	0.010 ^a	0.196 ^a	0.066	1.078	19.76	1.773	0.119
	L _L	0.005	0.034	0.040	0.881	17.88	1.346	0.076
	L _U	0.036	0.207	0.110	1.275	27.54	2.628	0.202
IV n=6	$\bar{x}_R$ $\bar{x}$	0.047 ^b	0.488 ^b	0.065	1.474	19.36	2.025	0.177
	L _L	0.029	0.004	ND	0.596	16.34	ND	ND
	L _U	0.067	0.876	0.360	2.402	26.46	10.64	0.415
L*		0,1	0,4	0,2	20	50	NL	2,0

n – number of samples

^{a,b} – means marked with different letters within a given heavy metal and a given area differ at P<0.05

L* – Hygienic limits for the content of heavy metals in foods (mg.kg⁻¹) Regulation No. 298/1997 of the Ministry of Health, Czech Republic

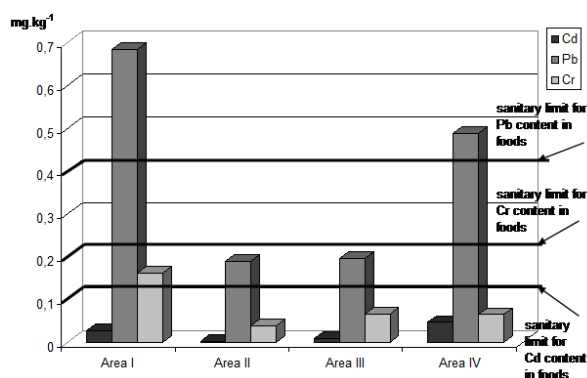
$\bar{x}_R$ – re-transformed mean (Box-Cox transformation) $\bar{x}$

L_L – lower limit in mg. kg⁻¹

L_U – upper limit in mg. kg⁻¹

NL – no limit

ND – not detected



2: Average values of Cd, Pb and Cr content in red fox intestines and hygienic limits for Cd, Pb and Cr content in foods.

(2010) in the Krušné Hory Mts (Czech Republic) where hygienic limits for Cd and Pb were exceeded in the kidneys of foxes.

Consistent with these ideas, Bilandžić *et al.* (2010) found that no heavy metal content in the kidneys of foxes exceeded hygienic limits in rural areas of central Croatia – Zagorje. However, other articles demonstrate the differences

between pollution in urban, suburban and rural areas conclude that despite deeply rooted assumptions, contamination may be higher in rural areas than in urban areas. For example Dip *et al.* (2001) have compared heavy metal concentrations in the tissues of red foxes from adjacent urban, suburban, and rural areas near Zürich. Higher contents of Cd were recorded

in the rural area. A major storage site for Cd in Switzerland is in the matrix of the upper soil layers. Exceeded hygienic standard for the content of Cd was observed in the kidneys of foxes from rural areas of Urbino-Pesaro (Italy) Alleva *et al.* (2006).

While Area IV in our work can be assessed as industrial agglomeration and some environmental damage can be expected, Area I has the character of a nature park without a significant local source of air pollution. Nevertheless, we detected the highest environmental pollution by heavy metals there, particularly of Pb and Cr. It is clear that long-term impact of emissions from the past applies here. Similarly, Millán *et al.* (2008) in Doñana National Park (Spain) found an increased presence of heavy metals in areas without local emission sources, where foxes' kidney content of heavy metals exceeded the hygienic limits for food according to Czech legislation (Cd 0.113 mg.kg⁻¹, Cu 72 mg.kg⁻¹ and Zn 136.7 mg.kg⁻¹). The explanation for this high content of heavy metals can be found in an ecological disaster 10 years before the monitoring.

The rate of environmental pollution is usually evaluated according to the current level of emissions in the air. The contents of heavy metals in atmospheric aerosol in Czech Republic are monitored by 62 stations of the Czech Hydrometeorological Institute. According to the Czech Hydrometeorological Institute, concentrations of atmospheric aerosol as well as the percentage of heavy metals have fallen from 1988, in accordance with the declining usage of solid fuels and the related decrease in

solid emissions especially from major sources of air pollution. The power stations Hirschfelde and Hagenwerde in Germany were dismantled before the year 1999 and power plants in North Bohemian Coal Field were modernized. In 2011, no station in the area recorded exceeded the limit for Pb (500 ng.m⁻³.year⁻¹), or Cd (5 ng.m⁻³.year⁻¹). At one station outside the observed area, the limit for Ni (20 ng.m⁻³.year⁻¹) was exceeded.

The results of Millán *et al.* (2008) and of our work show that the current state of emissions in the atmosphere does not say anything about possible environmental contamination by heavy metals. Due to environmental damage from the past, even a low level of air pollution may be caused – through accumulation of heavy metals in plants and animals – the exceeding of hygienic limits for the content of heavy metals in foods. Increased risk is connected with the slaughtering of animals with longer life length. The results of this study show that the northern part of Czech Republic is contaminated by heavy metals and the references cited in this work indicate that the problem of environmental contamination involves other European areas. Considering this fact, with respect to the transmission of emissions over long distances, pollution surveys should not be focused solely on the territory with air-pollution load.

In order to have safe food production the regular monitoring of heavy metal concentrations in the tissues of wild and domestic animals from all areas is necessary in the near future.

Conclusion

This work investigates the level of environmental contamination by heavy metals in countryside of the Czech Republic. The accumulation of these metals was measured by monitoring of their concentration in tissues of red foxes (*Vulpes vulpes*). Samples were collected during 2009–2010 in the northern part of the Czech Republic and were analyzed by ETA AAS (Pb, Cr, Ni, Cd) and FAAS (Cu, Mn, Zn). Results of this study indicate that urban, suburban and industrial areas show increased concentrations of heavy metals, especially the food consumption limits of Pb and Cr were exceeded. The limits of lead and chromium have been crossed in two studied areas, in Krasna Lipa and Hradec Kralove. Concentrations of Pb (0.686 mg.kg⁻¹) and Cr (0.162 mg.kg⁻¹) that exceed the hygienic limits for food were found in red foxes tissues.

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Contact information

Jana Sedláková: jani.sedl@seznam.cz