ACCUMULATION OF HEAVY METALS IN SELECTED ORGANS OF YELLOW-NECKED MOUSE (*Apodemus flavicollis*)

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Abstract

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Concentration of copper, iron, manganese, zinc and cadmium in liver, kidney, testis and uterus of *Apodemus flavicollis* in relation to sex and locality were investigated. For analysis of the content of these trace elements an AAS method was used.

In the organs of *A. flavicollis* from the area of power station Nováky almost in all cases higher concentration of observed elements than in the organs of animals from the area of nuclear power station Mochovce were detected.

The females of *A. flavicollis* from Nováky locality have shown significantly higher concentration of copper in uterus ($p < 0.05$) and in kidneys ($p < 0.01$), higher concentration of iron in kidneys ($p < 0.01$), higher concentration of zinc in kidneys ($p < 0.01$) and in liver ($p < 0.05$) and higher concentration of cadmium in kidneys ($p < 0.01$) and liver ($p < 0.01$) in comparison with females from Mochovce locality. A higher level of manganese was recorded ($p < 0.01$) in kidneys of the females from Mochovce (0.3047 $mg.kg^{-1}$). A higher concentration of zinc was found in the liver of the males from Nováky area ($p < 0.05$). On the contrary, there was significantly higher amount of zinc in the testis of *A. flavicollis* from Mochovce.

Key words: heavy metals, copper, iron, manganese, zinc, cadmium, *Apodemus flavicollis,* liver, kidney, testis, uterus

Introduction

Chemical production and burning of the fossils fuel is connected with the source of large amounts of toxic waste, especially sulphur oxides, nitric gasses and heavy metals emissions, which are toxic for all living organisms. Their danger has been related with food chain movement and with their ability to absorb and accumulate (Rous, Jelínek, 2000). The importance of monitoring and study of the heavy metal effects on living organisms has increased especially in the last decades. Their amount and sort has been increasing and is connected with gradual increase of their concentration in all part of environment.

Toxic elements from industrial fumes cause morphological changes and thus also physiological or genetic changes. Pathological changes are often related to blood and blood producing organs, genitals and reproduction, digestive tract and respiratory apparatus. There is a significant relation between the amount of metal residue in soil, water, also in food and in the organs of mammals and representatives of remaining classes of vertebrates, first of all in liver and kidneys (Pankakoski et al., 1993; Shore, 1995; Komarnicki, 2000).

Small ground mammals are also used as the bio-indicators of anthropogenic pollution of the landscape. They reach high abundance, in the areas exposed to industry and they have limited ability to migrate. Game rodents have small individual territories which enables detection of the local phenomenon (Flickinger, Nichols, 1990).

The purpose of the study was to determine concentration and distribution of copper, iron, manganese, zinc and cadmium in organs of *A. flavicollis* in relation to the sex and locality occurrence.

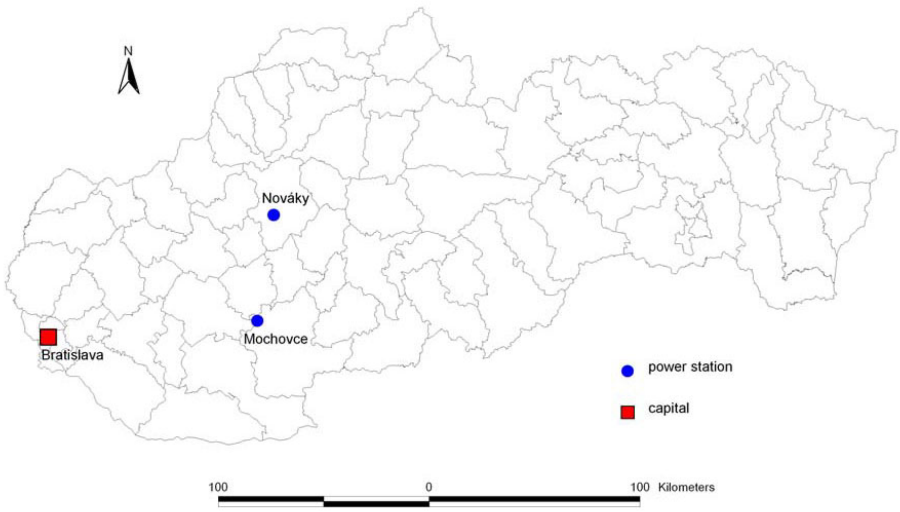

Fig. 1. Localities of power stations in the Slovak Republic (Central Europe).

Materials and methods

The individuals of yellow-necked mouse (*Apodemus flavicollis, Muridae,* Rodentia) were obtained by means of the standard teriological methods and procedures from wood ecosystems in surroundings of nuclear power station Mochovce and power station Nováky (the Slovak Republic, Central Europe; Fig. 1). All samples used in the experiment (7 males and 7 females Mochovce, 7 males and 7 females Nováky) were adult, in good physical condition, without pathological- anatomical changes.

The concentration of heavy metals: copper, iron, manganese, zinc and cadmium in liver, kidneys, testis and uterus were determined with method of atomic absorption spectrophotometry.

The tissue samples were kept at -18 °C until analysis. In the laboratory the samples (liver, kidney, testis, and uterus) were weighed (minimum 2 g) and ashed with diluted nitric acid p.a. ($HNO_3 : H_2O = 2 : 1$) at 130 °C for 2 h. Undisclosed particles were filtered off and the solution diluted to 25 ml. The concentrations of cooper, iron, manganese, zinc and cadmium were analyzed by AAS (Perkin – Elmer 4100 ZL) in a graphite furnance. All metal concentrations are expressed on a wet weight basis in $mg.kg^{-1}$.

From final data, basis statistical characteristics were calculated (mean, standard deviation, minimum, maximum, median), and an analysis of variance by Student's t-test was completed for each variable using PC program Statgraphics.

Results and discussion

Concentrations of cooper, iron, manganese, zinc and cadmium in male and female organs of *Apodemus flavicollis* from the surroundings of the nuclear power station Mochovce are listed in Table 1.

Copper concentration in liver was significantly ($p < 0.05$) higher in females (5.005 $mg.kg^{-1}$) in comparison with males (1.6393 $mg.kg^{-1}$). Significant ($p < 0.05$) difference was also found in copper levels in liver (5.005 $mg.kg^{-1}$) and in uterus (0.0077 $mg.kg^{-1}$). Gašparík et al. (2004) found significantly higher level of copper in liver in comparison with kidneys and muscles. According to Jenčík et al. (2000) genitals belong to organs with low level of copper. On the other hand Komarnicki (2000) describes higher accumulation of copper in females gonads. The ratio of *A. flavicollis* females liver and kidney contamination was 1.80:1. In males testes were the most contaminated organ, followed by kidneys (1.9105 $mg.kg^{-1}$) and liver (1.6393 $mg.kg^{-1}$).

The highest concentrations of iron were again found in uterus (471.733 $mg.kg^{-1}$). Lower content of iron was in liver (280.367 $mg.kg^{-1}$). The differences in contamination of analysed organs of males were minimal also in absolute values. The highest concentration of manganese was recorded in liver of males (0.731 $mg.kg^{-1}$). In remaining organs of males and females there was mean concentration of this microelement multiple times lower.

The mean levels of zinc in all organs of both sexes were relatively equal. Significant difference has been recorded only in females; the concentration of zinc is statistically higher ($p < 0.05$) in uterus (51.60 $mg.kg^{-1}$) in comparison with kidneys (21.31 $mg.kg^{-1}$). High concentration of zinc in female gonads has been also found (Komarnicki, 2000).

Cadmium is non-essential element with high potential ability to move in the food chain (Hunter et al., 1987). In all samples the levels of this high toxic metal were relatively low. In liver and kidneys any significant differences between sexes were recorded. In males there higher ($p < 0.05$) concentration in kidneys (0.0365 $mg.kg^{-1}$) when compared with liver

T a b l e 1. The concentration of selected trace elements in liver, kidney, testes and uterus in *Apodemus flavicollis* from Mochovce

Organ		Element									
		Cu [mg.kg⁻¹]		Fe [mg.kg⁻¹]		Mn [mg.kg⁻¹]		Zn [mg.kg⁻¹]		Cd [mg.kg⁻¹]	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver	$\bar{x}$	1.6493	5.0050	288.9750	280.3667	0.7305	0.2860	40.6878	33.4400	0.0108	0.0027
	sd	1.6409	4.9803	67.3614	156.8000	1.0115	0.4936	23.6037	17.2314	0.0066	0.0006
	min	0.0020	0.3840	213.8000	107.1000	0.0010	0.0010	5.8310	13.9000	0.0030	0.0020
	max	3.7990	10.280	370.1000	412.5000	2.1480	0.8560	55.7900	46.4600	0.0190	0.0030
	med	1.3780	4.3510	286.0000	321.5000	0.3865	0.0010	50.5650	39.9600	0.0105	0.0030
Kidney	$\bar{x}$	1.9105	2.7830	293.5000	231.2667	0.1553	0.3047	56.0700	21.3100	0.0365	0.0117
	sd	1.6173	0.8483	117.5791	74.1408	0.3039	0.5260	42.3231	5.8992	0.0180	0.0021
	min	0.6950	1.9480	174.5000	172.3000	0.0010	0.0010	29.3100	14.9100	0.0140	0.0100
	max	4.1540	3.6440	441.000	314.5000	0.6110	0.9120	118.700	26.5300	0.0570	0.0140
	med	1.3965	2.7570	279.2500	207.000	0.0045	0.0010	38.1350	22.4900	0.0375	0.0110
Testis	$\bar{x}$	3.9413	–	239.2250	–	0.0013	–	77.4903	–	0.0090	–
	sd	3.4672	–	80.6869	–	0.0005	–	72.2332	–	0.0034	–
	min	0.0440	–	168.6000	–	0.0010	–	39.6300	–	0.0040	–
	max	8.4870	–	355.0000	–	0.0020	–	185.800	–	0.0110	–
	med	3.6170	–	216.6500	–	0.0010	–	42.2655	–	0.0105	–
Uterus	$\bar{x}$	–	0.0077	–	471.7333	–	0.0103	–	51.6000	–	0.0077
	sd	–	0.0015	–	287.8126	–	0.0162	–	27.7230	–	0.0015
	min	–	0.0060	–	221.8000	–	0.0010	–	29.4500	–	0.0060
	max	–	0.0090	–	786.4000	–	0.0290	–	82.6900	–	0.0090
	med	–	0.0080	–	407.0000	–	0.0010	–	42.6600	–	0.0080

$\bar{x}$ – mean, sd – standard deviation, min – minimum, max – maximum, med – median

Cu: $p < 0.05$ (liver females *vs* males; females: liver *vs* uterus). Zn: $p < 0.05$ (females: uterus *vs* liver and kidney). Cd: $p < 0.01$ (females: kidney *vs* uterus), $p < 0.05$ (females: kidney *vs* liver, males: kidney *vs* liver and testis).

Cu: $p < 0.01$ (females: kidney *A. flavicollis* from Nováky *vs A. flavicollis* from Mochovce), $p < 0.05$ (females: uterus *A. flavicollis* from Nováky *vs A. flavicollis* from Mochovce). Fe: $p < 0.01$ (females: kidney *A. flavicollis* from Nováky *vs A. flavicollis* from Mochovce). Mn: $p < 0.01$ females: kidney *A. flavicollis* from Nováky *vs A. flavicollis* from Mochovce). Zn: $p < 0.01$ (females: kidney *A. flavicollis* from Nováky *vs A. flavicollis* from Mochovce, males: testis *A. flavicollis* from Mochove *vs A. flavicollis* from Nováky), $p < 0.05$ (liver males and females *A. flavicollis* from Nováky *vs A. flavicollis* from Mochovce). Cd: $p < 0.01$ (females: kidney and liver *A. flavicollis* from Nováky *vs A. flavicollis* from Mochovce)

(0.0108 mg.kg^{-1}) and with testis (0.009 mg.kg^{-1}) is reported. Analogous situation occurred also in females. Significantly higher level of cadmium in kidneys is given by Gašparík et al. (2004). Significantly higher concentration has been found in kidneys (0.0117 mg.kg^{-1}) in comparison with uterus (0.0077 mg.kg^{-1}) and liver (0.0027 mg.kg^{-1}; $p < 0.05$). Toman, Massányi (2002) regard the evaluation of cadmium content in gonads as the important factor in judging fertility disorders.

The levels of metals analysed in male and female organs of *A. flavicollis* from areas of power station Nováky are presented in Table 2.

The highest concentration of copper was found in uterus of *A. flavicollis* (10.264 mg.kg^{-1}). In liver, kidneys and gonads of males the concentrations of copper were lower. The ratio of uterus and kidneys or liver contamination of female *A. flavicollis* was 1.91:1, or 2:1. In males liver was the most contaminated organ (7.565 mg.kg^{-1}). Bukovjan et al. (1997) report the copper concentration in liver of *Lepus europaeus* 4.683 mg.kg^{-1}, which is nearly half of the value that we have found. In kidneys the values are comparable with our results.

The highest concentration of iron was found in uterus (618.5 mg.kg^{-1}). The lower content was found in kidneys (506.9 mg.kg^{-1}) and in liver (455.233 mg.kg^{-1}). In males, liver

T a b l e 2. The concentration of selected trace elements in liver, kidney, testes and uterus in *Apodemus flavicollis* from Nováky

Organ		Element									
		Cu [mg.kg⁻¹]		Fe [mg.kg⁻¹]		Mn [mg.kg⁻¹]		Zn [mg.kg⁻¹]		Cd [mg.kg⁻¹]	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver	$\bar{x}$	7.565	5.133	445.000	455.233	0.887	0.748	101.064	48.477	0.100	0.023
	sd	6.934	1.788	161.490	192.398	1.412	0.891	40.191	13.876	0.105	0.004
	min	2.334	3.239	155.300	276.100	0.001	0.004	55.470	38.970	0.003	0.019
	max	22.320	6.693	589.400	658.600	3.019	1.735	167.600	64.400	0.260	0.027
	med	5.654	5.768	493.000	431.000	0.080	0.504	95.310	42.060	0.055	0.023
Kidney	$\bar{x}$	4.816	5.376	354.986	506.900	0.631	0.055	81.637	85.863	0.086	0.071
	sd	3.653	1.204	95.457	65.574	0.618	0.090	53.384	18.855	0.128	0.031
	min	0.175	3.997	215.100	434.500	0.084	0.001	47.430	67.530	0.011	0.035
	max	9.037	6.216	470.900	562.300	1.876	0.159	200.00	105.200	0.370	0.094
	med	3.263	5.915	338.000	523.900	0.377	0.004	62.190	84.860	0.049	0.083
Testis	$\bar{x}$	3.2163	–	259.143	–	0.171	–	51.380	–	0.083	–
	sd	1.510	–	123.859	–	0.367	–	12.209	–	0.095	–
	min	1.018	–	118.600	–	0.001	–	34.170	–	0.008	–
	max	5.413	–	439.000	–	0.997	–	72.110	–	0.260	–
	med	3.450	–	283.400	–	0.009	–	48.650	–	0.042	–
Uterus	$\bar{x}$	–	10.264	–	618.500	–	2.272	–	91.140	–	0.018
	sd	–	8.839	–	365.982	–	3.379	–	49.638	–	0.011
	min	–	0.891	–	195.900	–	0.004	–	43.550	–	0.006
	max	–	18.450	–	830.400	–	6.156	–	142.600	–	0.025
	med	–	11.450		829.200	–	0.656	–	87.270	–	0.024

$\bar{x}$ – mean, sd – standart deviation, min – minimum, max – maximum, med – median

Fe: $p < 0.01$ (kidney females *vs* males), $p < 0.05$ (males: liver *vs* testis, kidney *vs* testis). Zn: $p < 0.01$ (females: kidney *vs* liver), $p < 0.05$ (liver males *vs* females, liver males *vs* testis). Cd: $p < 0.01$ (females: kidney *vs* uterus, kidney *vs* liver, liver *vs* uterus)

was the most contaminated organ (445 mg.kg^{-1}). Its contamination was significantly ($p < 0.05$) higher in comparison with testis. Statistically higher level of iron was in kidneys of males than in testis. Significantly higher level of iron was in kidneys of females when compared with males ($p < 0.01$).

Relatively high levels of manganese were found in the samples of uterus ($\bar{x}$= 2.272 mg.kg^{-1}; maximum value 6.156 mg.kg^{-1}). In liver and kidneys of both sexes and in testis the mean concentrations were multiple times lower.

The high mean values of zinc were found in all examined organs, especially when compared with results of Bukovjan et al. (1997). Comparable values described Komarnicki (2000) in *Talpa europaea.* The highest level of the element was recorded in liver of the males (101.064 mg.kg^{-1}). In comparison with females we found significantly higher level of this element ($p < 0.05$) in males. The higher concentration of zinc ($p < 0.05$) was in liver of the males than in testis. Concentration of zinc in females kidneys was significantly higher ($p < 0.01$) than concentration in liver.

Cadmium content in parenchymatosis organs showed low values. Concentrations found in liver correlate with former analyses (Jančová et al., 2002). Content of cadmium in kidneys in comparison with quoted study has fallen. Kidney is the main organ where cadmium is accumulated in normal conditions. In acute intoxication the largest amount of the metal is not accumulated by kidneys but by liver and from liver, cadmium bound with metallothionein is redistributed to other organs as well as in kidneys where this complex is fixed and cadmium is accumulated there. Majority of the authors describe the highest concentrations of cadmium in kidneys (Toman, Massányi, 1996; Massányi et al., 2003) and our results also correspond with them. The ratio of kidney and liver contamination in males was 1:1.163 and in females 3.087:1. Cadmium concentration in kidneys of the females is significantly higher ($p < 0.01$) than concentration in liver and uterus. Significant difference ($p < 0.05$) was found when liver and uterus had been compared. Slamečka et al. (1994) described in liver of hares from different localities in Slovakia higher values (0.217–0.523 $mg.kg^{-1}$). According to Komarnicki (2000) kidneys are the most contaminated and they are followed by liver.

The average concentration of copper in uterus of *Apodemus flavicollis* from locality Nováky (10.264 $mg.kg^{-1}$) is significantly higher ($p < 0.05$) than in Mochovce (0.0077 $mg.kg^{-1}$). When comparing copper level in kidneys of the females we found statisticant difference ($p < 0.01$). Female kidneys from locality Nováky were contaminated by significantly higher amount of copper than female kidneys from locality Mochovce.

Concentration of iron in females' kidneys from area Nováky is higher than in females from area Mochovce ($p < 0.01$).

In manganese we recorded higher level ($p < 0.01$) in females kidneys from Mochovce (0.3047 $mg.kg^{-1}$).

Zinc is an essential trace element. It is necessary for all species of living organisms. It is a part of more than 200 metal-enzymes and thus influences living important functions. Its toxicity is low and not determined for individual animal species. In general, the younger individuals are considered to be more sensitive to the increased amounts of zinc. From therapeutic aspect, it is important that zinc does not belong to cumulative toxins in contrast with cadmium, mercury and lead. When the content of zinc had been compared, the statistically important differences were found in both of the sexes. The females from locality Nováky had higher level of this element in kidneys ($p < 0.01$) and in liver ($p < 0.05$). More zinc was accumulated in liver of males from area Nováky ($p < 0.05$). It is a paradox that significantly higher amount of zinc was in testis *A. flavicollis* from Mochovce.

In kidneys of females from locality Nováky we found significantly ($p < 0.01$) higher amount of cadmium than in kidneys of females from Mochovce. The same conclusion can be applied for cadmium in liver. Liver and kidney contamination of the males from Mochovce was lower in comparison with individuals from the area of power station, but the differences were not significant.

Conclusions

The females of *A. flavicollis* from Nováky locality have shown significantly higher concentration of copper in uterus ($p < 0.05$) and in kidneys ($p < 0.01$), higher concentration of iron in kidneys ($p < 0.01$), higher concentration of zinc in kidneys ($p < 0.01$) and in liver ($p < 0.05$) and higher concentration of cadmium in kidneys ($p < 0.01$) and liver ($p < 0.01$) in comparison with females from Mochovce locality. A higher level of manganese was recorded ($p < 0.01$) in kidneys of the females from Mochovce (0.3047 $mg.kg^{-1}$). A higher concentration of zinc was found in the liver of the males from Nováky area ($p < 0.05$). On the contrary, there was significantly higher amount of zinc in the testis of *A. flavicollis* from Mochovce.

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Jančová A., Massányi P., Naď P., Koréneková B., Skalická M., Drábeková J., Baláž I.: **Akumulácia ťažkých kovov vo vybratých orgánoch ryšavky žltohrdlej (*Apodemus flavicollis*).**

Metódou atómovej absorpčnej spektrofotometrie (AAS) sme stanovili koncentrácie ťažkých kovov: medi (Cu), železa (Fe), mangánu (Mn), zinku (Zn) a kadmia (Cd) v pečeni, obličkách, semenníkoch, prisemenníkoch a maternici ryšavky žltohrdlej (*Apodemus flavicollis*) v závislosti od pohlavia a lokality výskytu.

V orgánoch *Apodemus flavicollis* z oblasti Tepelnej elektrárne Nováky sme takmer vo všetkých prípadoch zistili vyššie koncentrácie sledovaných prvkov ako v orgánoch jedincov z oblasti Jadrovej elektrárne Mochovce.

Samice *Apodemus flavicollis* z lokality Nováky vykazujú signifikantne vyššiu koncentráciu medi v maternici ($p < 0{,}05$) a v obličkách ($p < 0{,}01$), vyššiu koncentráciu Fe v obličkách ($p < 0{,}01$), vyššiu koncentráciu Zn v obličkách ($p < 0{,}01$) i v pečeni ($p < 0{,}05$) a vyššiu koncentráciu Cd v obličkách ($p < 0{,}01$) a v pečeni ($p < 0{,}01$) v komparácii so samicami z lokality Mochovce. Vyššiu hladinu Mn sme zaznamenali ($p < 0{,}01$) v obličkách samíc z Mochoviec (0,3047 $mg.kg^{-1}$). Viac Zn akumulovala pečeň samcov z oblasti Nováky ($p < 0{,}05$). Naopak, signifikantne vyššie množstvo Zn bolo v semenníkoch *Apodemus flavicollis* z Mochoviec.