

Heavy Metals in Brown Bears from the Central European Carpathians

O. ČELECHOVSKÁ¹, I. LITERÁK¹, S. ONDRUŠ², Z. POSPÍŠIL¹

¹University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

²Administration of the National Park Low Tatras, Banská Bystrica, Slovakia

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Abstract

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The aim of the present study was to assess heavy metal load in the brown bear (*Ursus arctos*) living in the central European Carpathians. Between 2002 and 2004, fifteen bears were examined to evaluate the distribution of cadmium, lead, mercury and copper in the animals' muscles (diaphragm), spleen, liver and kidney. The highest content of cadmium, lead and mercury was found in the kidney ($17.4 \pm 5.2 \text{ mg}\cdot\text{kg}^{-1}$, $1.16 \pm 0.39 \text{ mg}\cdot\text{kg}^{-1}$, $0.39 \pm 0.25 \text{ mg}\cdot\text{kg}^{-1}$), whereas the lowest content of the metals was observed in the muscles ($0.017 \pm 0.009 \text{ mg}\cdot\text{kg}^{-1}$, $0.299 \pm 0.308 \text{ mg}\cdot\text{kg}^{-1}$, $0.013 \pm 0.011 \text{ mg}\cdot\text{kg}^{-1}$). Second highest concentration of cadmium, lead and mercury was detected in the liver ($0.83 \pm 0.24 \text{ mg}\cdot\text{kg}^{-1}$, $0.99 \pm 0.61 \text{ mg}\cdot\text{kg}^{-1}$, $0.11 \pm 0.05 \text{ mg}\cdot\text{kg}^{-1}$). Copper distributions and concentrations in bear tissues were as follows (in descending order): liver ($23.9 \pm 6.7 \text{ mg}\cdot\text{kg}^{-1}$), > kidneys ($9.0 \pm 3.3 \text{ mg}\cdot\text{kg}^{-1}$), > muscles ($1.9 \pm 1.6 \text{ mg}\cdot\text{kg}^{-1}$) and > spleen ($1.0 \pm 0.2 \text{ mg}\cdot\text{kg}^{-1}$). As compared with heavy metal load observed in bear tissues between 1988 and 1990, the concentration of cadmium in the muscles and liver was significantly lower ($p < 0.01$). On the contrary, mercury values were significantly higher in the liver ($p < 0.05$), kidney and muscle ($p < 0.01$). Lead and copper tissue concentrations did not change substantially.

Cadmium, Lead, Mercury, Copper, AAS, the Low Tatras

Heavy metals are a part of the Earth's crust and their biosphere incidence, distribution and form are influenced by anthropogenic activities rather than natural processes (Nriagu 1988, 1991; Nriagu and Pacyna 1988). Once released in the biosphere, metals persist for a long time while they may change their forms and dissolve (MacDonald et al. 2000). Immissions caused by burning fossil fuels and waste, car exhausts and residua of widely used pesticides can result in the environment contamination by inorganic and organic compounds. Chemical contamination of the environment impairs ecological balance. Sensitive plant species are affected, large forest areas are destroyed and toxic substances enter the food chain (Yaaqub et al. 1991; Gnamus et al. 1995; 2000). Even an insignificant concentration of these substances can, in the long run, endanger the health of the flora and fauna (Yaaqub et al. 1991; Massanyi et al. 2000ab; Beiglböck et al. 2002; Hell et al. 2005; Massanyi et al. 2005). Some wild animal species, especially predators representing the last component of the food chain, can be seen as bioindicators of environmental contamination by inorganic and organic pollutants (Gufner et al. 1997; Dietz et al. 2000; Hoekstra et al. 2003; MacDonald et al. 2005).

One of the top predators with a typical food strategy is the brown bear (*Ursus arctos*), living in Eurasian and North American areas that have been almost untouched by human activities. The bears of Central Europe live mainly in the Carpathians. Although inorganic and organic tissue contaminants in polar bears from various polar areas have been described relatively sufficiently (Dietz et al. 2000; Muir et al. 1999; Hoekstra et al. 2003; et al. 2001ab), heavy metal tissue concentrations of the brown bear have been studied only sporadically. Heavy metals in brown bear tissues were detected in the Karelian area of

Address for correspondence:

Doc. Ing. Olga Čelechovská, Ph.D.
Department of Biochemistry, Chemistry and Biophysics
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences
Palackého 1-3, 612 42 Brno, Czech Republic

Phone + 420 541 562 606
E-mail: celechovskao@vfu.cz
<http://www.vfu.cz/acta-vet/actavet.htm>

Russia (Medvedev 1999) and in the western Carpathians of Slovakia (Chudík and Maňkovská 1989; Žilínčar et al. 1992). Heavy metal contamination in the Slovak Carpathians can be influenced by industrial plants affecting the local environment for the past several decades. They are mainly thermal power stations consuming brown coal (Keegan et al. 2006), copper and aluminium works (Wilcke et al. 1998, 1999), or extractive industry (Míadoková et al. 2000). A direct connection between air pollution and contamination of animals was reported in previous studies (Tataruch and Onderschek 1991; Chudík and Maňkovská 1987).

The aim of our study was to determine the content and distribution of cadmium, lead, mercury and copper in tissues of brown bears living in the western Carpathians of central Slovakia between 2002 and 2004, to compare heavy metal concentrations with the values observed in the same location between 1988 and 1990 (Žilínčar et al. 1992), and to comment on the overall dynamism.

Materials and Methods

We examined tissue samples of 15 brown bears (*Ursus arctos*) from the orographic area of the Low Tatras mountain range. The Low Tatras, located in central Slovakia, make a central part of the western Carpathians. Tissues were collected from bears that were shot down during regulatory shooting (approved by the Ministry of the Environment of the Slovak Republic) in the autumn of 2002 (6 bears), 2003 (2 bears) and 2004 (7 bears). Sex distribution, body weight and age data are presented in Table 1. Samples of muscle (diaphragm), liver, spleen and kidneys were collected immediately after the shooting (no later than 12 hours after the death). Each sample was stored in a separate plastic bag at -18°C in a freezing box until the analysis was performed.

Table 1. Characteristics of examined bears

Sex	n	Weight (kg)		Age (year) y ± s.d.
		m ± s.d.	min - max	
F	10	108 ± 26	67.5 - 158	5.7 ± 1.6
M	5	117 ± 41	53 - 175	5.7 ± 2.4

Mercury, cadmium, lead and copper tissue concentrations were estimated. Mercury concentration was determined using dedicated AMA 254 mercury analyzer (Altech Ltd., Czech Republic). Cadmium, lead and copper detection was performed using flameless Atomic absorption spectroscopy (AAS) on Z-5000 appliance (Perkin Elmer Corp, USA). Detection limits of individual elements (3σ) and certified reference materials (SRM 1577b - bovine liver, BCR No 184 - bovine muscle, BCR No 186 - pig kidney) used for the verification of accuracy and suitability of the methods are described in Table 2. Pre-analytic preparation was based on the dissolution of samples by a mixture of nitric acid and hydrogen peroxide in a high-pressure laboratory microwave autoclave Uniclever (Plasmatronika, Poland). Means, standard deviations, Pearson correlation and separate variance *t*-test were carried out using statistical package Unistat 5 version 5.1.

Table 2. Limit of detection of method (LOD) and element concentration ± standard deviation in standard reference material of bovine liver (SRM 1577 b) and bovine muscle (BCR No 184).

Element	LOD µg·kg ⁻¹	SRM 1577b		BCR No. 184	
		Found mg·kg ⁻¹	Certified mg·kg ⁻¹	Found mg·kg ⁻¹	Certified mg·kg ⁻¹
Cd	1.02	0.511 ± 0.039	0.500 ± 0.030	0.012 ± 0.003	0.013 ± 0.002
Pb	9.31	0.133 ± 0.005	0.129 ± 0.004	0.234 ± 0.011	0.239 ± 0.011
Cu	70.4	163 ± 6	160 ± 8	2.30 ± 0.08	2.36 ± 0.06
Hg	0.01*	-	-	0.0031 ± 0.0003	0.0026 ± 0.0006

* Limit of detection of AMA-254

Results and Discussion

Mean concentrations in native tissues, standard deviations, medians and concentration ranges of cadmium, lead, mercury and copper in male and female bears are presented in Table 3. Significant differences between the males and females and individual elements (Cd, Pb, Hg, and Cu) were not found. This fact corresponds with the result described by Medvedev (1999).

Table 3. Mean element concentrations ($c \pm$ standard deviation), range and median in native tissues of bears

Element	Tissue	n	$c \pm$ s.d.	$C_{min} - C_{max}$	Median
			mg·kg ⁻¹		
Cd	muscle	9	0.017 ± 0.009	0.009 – 0.042	0.015
	spleen	12	0.072 ± 0.024	0.031 – 0.112	0.073
	liver	12	0.829 ± 0.240	0.58 – 1.24	0.782
	kidney	7	17.36 ± 5.22	9.08 – 27.32	16.44
Pb	muscle	9	0.299 ± 0.301	0.068 – 0.932	0.162
	spleen	12	0.165 ± 0.062	0.10 – 0.34	0.142
	liver	12	0.990 ± 0.609	0.41 – 2.62	0.767
	kidney	7	1.158 ± 0.391	0.65 – 1.73	1.235
Cu	muscle	9	1.91 ± 1.57	0.92 – 4.88	1.13
	spleen	12	0.97 ± 0.22	0.70 – 1.47	0.88
	liver	12	23.9 ± 6.72	12.2 – 39.3	24.3
	kidney	7	9.0 ± 3.29	4.62 – 14.01	7.82
Hg	muscle	9	0.013 ± 0.011	0.0003 – 0.04	0.010
	spleen	12	0.017 ± 0.012	0.001 – 0.025	0.014
	liver	12	0.107 ± 0.048	0.031 – 0.172	0.098
	kidney	7	0.386 ± 0.250	0.103 – 0.699	0.251

Table 4. Proportional representation of elements in tissues

Element	Cd	Pb	Hg	Cu
Tissue	% ± s.d.			
kidney	100	100	100	37 ± 11
liver	6.1 ± 1.4	94 ± 30	46 ± 17	100
spleen	0.53 ± 0.09	20 ± 4	8.7 ± 3.0	6.0 ± 1.2
muscle	0.10 ± 0.04	57 ± 9	5.9 ± 1.8	5.3 ± 2.6

Table 4 shows the distribution of individual elements in various tissues. Metal tissue distribution was assessed as an average representation (in %) of concentration of each element in the native tissue as compared with the tissue showing the highest concentration of the same element in each individual animal (\pm standard deviation). Kidney basic concentration (100%) was selected for cadmium, lead and mercury; copper distribution was assessed in liver.

Cadmium

Cadmium showed the most marked, highly significant differences ($p < 0.001$) in concentration among individual tissues (Tables 3 and 4). The highest cadmium concentration was found in the kidney, the lowest concentration (1000 × lower) was observed in the muscle (diaphragm). Lower concentration was determined in the liver and spleen. Cadmium accumulation in the kidney and liver has been reported also in other mammals (Braune et al 1999; Muir et al. 1999; Kottferová and Koréneková 2000; Beiglbock et al. 2002; Gašparik et al. 2004; Kramarová et al. 2005).

Cadmium concentrations determined in bear kidneys shot down between 2002 and 2004 were not statistically different from values described in bears examined between 1988 and 1990 (Žilinčar et al. 1992). However, the cadmium content in the liver and muscle (diaphragm) was significantly lower ($p < 0.01$).

Lead

The highest lead concentrations were found in the kidney. Concentration values in the liver were not significantly different from the kidney values. Highly significant difference in lead concentrations was observed between the kidney and muscle (diaphragm) ($p < 0.001$), kidney and spleen ($p < 0.001$), liver and muscle (diaphragm) ($p < 0.005$), and liver and spleen ($p < 0.001$). We found a significant correlation between the liver and kidney ($r = 0.70$, $p < 0.05$).

Changes of lead concentration in the muscle (diaphragm), liver and kidney as compared to values reported between 1988 and 1990 were not significant.

Mercury

A correlation between the body weight and mercury content was found in the kidney ($r = 0.89$, $p < 0.01$), which is in accordance with the cumulative function of mercury in the organism. Mercury cumulated in the tissues in the following order: kidney > liver > spleen > muscle (diaphragm). A significant difference in the mercury concentration was observed between diaphragm and spleen ($p < 0.05$) and liver and kidney ($p < 0.05$), while a highly significant difference was determined between the muscle (diaphragm) and liver, spleen and liver, muscle (diaphragm) and liver ($p < 0.001$), and spleen and kidney ($p < 0.01$).

It should be emphasized that mercury concentration in the liver was significantly higher ($p < 0.05$) compared to values determined between 1988 and 1990; the difference between the kidney and diaphragm was even higher ($p < 0.01$).

Copper

A significant correlation between the copper concentration in the spleen and the age of bears ($r = 0.88$, $p < 0.01$) was estimated. Copper is an essential element that is a part of many enzymes. The highest concentrations were observed in the liver, followed by the kidney, spleen and muscle (diaphragm), respectively. Highly significant concentration differences were observed between the liver and spleen, spleen and kidney, liver and kidney, muscle (diaphragm) and liver ($p < 0.001$), and kidney and muscle (diaphragm) ($p < 0.005$). Copper concentration in the spleen was not significantly different from the muscle (diaphragm) values.

The copper content in the kidney and muscle (diaphragm) was identical with the values published by Žilinčar et al. (1992). The concentration in the liver was significantly higher ($p < 0.01$) compared to Žilinčar et al. (1992) but identical with values reported by Medvedev (1999).

It is alarming that cadmium and lead concentrations determined in the kidney and liver of examined bears are still too high, mercury levels have even increased compared to values determined between 1988 and 1990. Relatively high concentrations of toxic elements were found also in tissues of foxes, boar (Piskorová et al. 2003) and deer (Gašparik et al. 2004). However, these levels are lower than those observed in bears and are in accordance with the assumption that the heavy metal load of omnivores is higher than that of ruminants living in the same area (Chudík and Maňkovská 1987). Although emissions of toxic elements have been decreasing since 1990 (Keegan et al. 2006), they are more acid and the mean soil acidification (Schwartz et al. 1999) allows for easier infiltration of certain metals into the soil extract and thus into plants. Thus metals become a part of the food chain of bears and other animals. This is also supported by recent reports on high heavy metal concentrations in mushrooms growing in the Spiš area, Slovakia (Zimmermannová

et al. 2001). Indeed, an increasing synanthropy of the western Carpathians bears can affect the heavy metal content in their tissues (Langgemacht et al. 1995).

Těžké kovy ve tkáních medvěda hnědého z Karpat

Cílem předkládané práce bylo zhodnotit zatížení medvěda hnědého (*Ursus arctos*) ze západních Karpat těžkými kovy. V letech 2002-2004 bylo vyšetřeno 15 medvědů na obsah kadmia, olova, rtuti a mědi a byla vyhodnocena distribuce kovů do svaloviny (bránice), sleziny, jater a ledvin. Nejvyšší koncentrace kadmia, olova a rtuti byly v ledvinách ($17,4 \pm 5$, $2 \text{ mg}\cdot\text{kg}^{-1}$, $1,16 \pm 0,39 \text{ mg}\cdot\text{kg}^{-1}$, $0,39 \pm 0,25 \text{ mg}\cdot\text{kg}^{-1}$), nejnižší ve svalovině ($0,017 \pm 0,009 \text{ mg}\cdot\text{kg}^{-1}$, $0,299 \pm 0,308 \text{ mg}\cdot\text{kg}^{-1}$, $0,013 \pm 0,011 \text{ mg}\cdot\text{kg}^{-1}$). Druhé nejvyšší koncentrace kadmia, olova a rtuti byly nalezeny játrech ($0,83 \pm 0,24 \text{ mg}\cdot\text{kg}^{-1}$, $0,99 \pm 0,61 \text{ mg}\cdot\text{kg}^{-1}$, $0,11 \pm 0,05 \text{ mg}\cdot\text{kg}^{-1}$). Distribuce mědi do tkání medvěda byla v sestupném pořadí koncentrace: játra ($23,9 \pm 6,7 \text{ mg}\cdot\text{kg}^{-1}$) > ledviny ($9,0 \pm 3,3 \text{ mg}\cdot\text{kg}^{-1}$) > svalovina ($1,9 \pm 1,6 \text{ mg}\cdot\text{kg}^{-1}$) > slezina ($1,0 \pm 0,2 \text{ mg}\cdot\text{kg}^{-1}$). Ve srovnání se zjištěnými koncentracemi těžkých kovů ve tkáních medvědů z období 1988-1990 byly koncentrace kadmia ve svalovině a játrech významně nižší ($p < 0,01$), u rtuti došlo naopak ke statisticky významnému zvýšení u jater ($p < 0,05$), ledvin a svaloviny ($p < 0,01$). U olova a mědi nedošlo k podstatným změnám v koncentracích ve tkáních.

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