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HEAVY METAL LEVELS IN THE TISSUES OF WILD LIVING ANIMALS FROM TWO DISTINCT INDUSTRIALLY EXPLOITED AREAS IN SLOVAKIA

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ABSTRACT

The aim of the presented study was to assess the heavy metal burden in biotopes of wild living animals of two distinct industrially exploited areas in Slovakia. 411 samples of various tissues (lung, liver, kidney, spleen, heart and muscle) of red deer, roe deer, mouflon, chamois, wild boar, European brown hare, fox, European brown marten, European badger, gray wolf, brown bear, wildcat, red squirrel, European polecat, alpine marmot, and European otter were collected from the localities between 2014 and 2018. Concentrations of mercury, cadmium, lead, arsenic, nickel, copper and zinc were determined using Atomic absorption spectroscopy. Significant correlations ($p < 0.05$, $t = 0.03162$) of metal levels in each locality and differences between the animals species were recorded. We have found important heavy metal burden in a relatively clean area – Tatra National Park that is legislatively protected and restricted in any industrial activity. In the Zemplín region, the examined heavy metal levels confirm permanent pollution by intensive heavy industrialization. Mostly mercury (29 %) was the metal that exceeded the legal limits permitted for human consumption, then cadmium (28 %) and lead (23 %). Concentration of chromium did not exceed the limit in any sample. The most burdened animal species was wild boar.

Key words: heavy metal; wild animal; tissue; environment; contamination; plant

INTRODUCTION

Animals act as very important part of substance and energy circulation and they play an important role in ecological stabilization of ecosystems as well. However the role of animals is often underestimated (Kulhavý *et al.*, 2003). Contaminants in wild living animals in Slovakia have been monitored since 1995. The basic aim of monitoring is to have a review of the levels and penetration of substances in selected game and fish species. As animals live in various types of biotope and belong to the primary consumer group, data from this study could be evaluated as an appropriate bioindicator of the actual state of the environment and the ecological balance. Thus we can also notice more information about food products – venison and fish (Krížová and Šalgovičová, 2002).

Metals, which are able to cumulate in soil, vegetation and other living organisms, belong to important environment contaminants. Generally, metals do not undergo chemical degradation but are cumulated in upper soil layers. Progressive transport of metals from soil to plants causes higher concentration in animal tissues (Gallo, 1995). Increased heavy metal levels in animal organs and tissues are induced by respiration

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from air and contaminated food intake. Other sources of environmental pollution are industrial fertilizers, exhaust gas from traffic, urban waste, etc. (Kováč *et al.*, 2005; EFSA 2010; Küttner *et al.*, 2014). The highest metal contamination risk is in the surroundings of metal industries, electric power stations and cement mills, which pollute soil and air with air pollutants and those subsequently pass into the food chain. In comparison with domestic animals wild living animals are influenced by the environmental conditions over the whole year (Niemi *et al.*, 1993; Tataruch, 1995; Kugonič and Zupan, 1999). Metals are cumulated by food because of their solubility and mobility that can cause serious ecological and health danger (Abu Al-Rub *et al.*, 2004). Heavy metal monitoring is very important not only for game but also for humans. Permanent exposure of organism to mildly increased concentrations of metals in environmental components is an actual problem for the human population, especially that living in industrial agglomerations. Chronic professional exposure is hardly diagnosed, the symptoms are not specific. Mostly it manifests as balance disorders of organism and chronic multi-symptomal stages – civilization diseases. In some patients, primary diseases are exacerbated by an increased metal concentration in the organism. Game constantly living in natural conditions is a very important bioindicator of its real pollution situation. Examination of wild living animals is the best way to know the level of heavy metal contamination in the natural environment (Babička and Sedláček, 2000).

In Slovakia, legal limits of heavy metal levels in animal body tissues that are acceptable for human consumption are defined in Food Codex of Ministry of Agriculture of Slovak Republic (Regulation of the Ministry of Agriculture and Rural Development of the Slovak Republic and the Ministry of Health of the Slovak Republic from 11 September 2006 No. 18558/2006-SL.).

The aim of our study was to determine the content of mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), nickel (Ni), copper (Cu) and zinc (Zn) in the tissues of various species of wild living animals from two different industrially exploited areas in Slovakia, to compare the metal contamination burden between the two areas and among the game species and to evaluate the actual situation of the environmental pollution in the localities.

MATERIAL AND METHODS

Tissue samples of various kinds of game species were examined to detect concentrations of heavy metals (mercury, cadmium, lead, arsenic, nickel, copper and zinc) cumulated in animal organisms from two parts of Slovakia (Figure 1). The first locality was Tatra National Park (TANAP) situated in the central north of Slovakia bordering on Poland. It is known as a legislatively protected area. The second locality, Zemplin region in eastern Slovakia, is characterised by its rich industrial exploitation.

Figure 1. Map of Slovakia monitored areas – Tatra National Park (A) and Zemplin region (B)

Table 1. The number of samples from game species in Tatra National Park and Zemplin

Animal species	n (Number of samples)	
	TANAP	Zemplín
Wild boar (*Sus scrofa*)	45	13
Red deer (*Cervus elaphus*)	41	30
Fox (*Vulpes vulpes*)	17	20
Roe deer (*Capreolus capreolus*)	11	19
Gray wolf (*Canis lupus*)	14	13
European badger (*Meles meles*)	-	5
Wildcat (*Felis silvestris*)	-	14
Mouflon (*Ovis musimon*)	-	18
Brown hare (*Lepus europaeus*)	-	57
Chamois (*Rupicapra rupicapra tatrica*)	13	-
European brown marten (*Martes martes*)	12	-
Brown bear (*Ursus arctos*)	26	-
Alpine marmot (*Marmota marmota*)	10	-
European polecat (*Mustela putorius*)	9	-
Red squirrel (*Sciurus vulgaris*)	9	-
European otter (*Lutra lutra*)	15	-
Total	222	189

During the huting seasons 2014/2015 – 2017/2018, tissues from hunted or dead animals – red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), mouflon (*Ovis musimon*), chamois (*Rupicapra rupicapra tatrica*), wild boar (*Sus scrofa*), European brown hare (*Lepus europaeus*), fox (*Vulpes vulpes*), European brown marten (Martes martes), European badger (*Meles meles*), gray wolf (*Canis lupus*), brown bear (*Ursus arctos*), wildcat (*Felis silvestris*), red squirrel (*Sciurus vulgaris*), European polecat (*Mustela putorius*), alpine marmot (*Marmota marmota*), and European otter (*Lutra lutra*) – were collected. Twelve species of wild animals were examined in TANAP and nine species from Zemplin. In total, we gained 411 of various kinds of tissue – lung, liver, kidney, spleen, heart and muscle, 222 samples from TANAP and 189 from Zemplin (Table 1). It was not possible to obtain exactly the same type of tissue and number in all animal species. The samples did not have any pathological lesions and were without toxic lead ammunition (weight from 50 g to 200 g). Each sample was stored in a plastic bag at -18 °C in a freezing box until the laboratory test was performed.

In 2018, due to the observed elevated levels in the wildlife samples, the samples of biotope components (bark of trees, leaf litter, needles, moss, grass) were taken from the TANAP area and the concentrations of the measured elements were then determined for the comparison. The samples were taken at the same sites where the sampling was carried out. Sample preparation consisted of thawing, homogenizing by means of a laboratory mixer, a laboratory mill and a laboratory mortar.

After preparation of macerate and wet mineralization with HNO_3 in MDS 2000 pressure microwave oven, sample filtrates were used to estimate heavy metal concentrations.

After the macerate preparation and mineralization, we used the sample filtrates to estimate heavy metal concentrations. Mercury level was determined using Atomic absorption spectroscopy (AAS) with dedicated AMA mercury analyzer (Advance Mercury Analyzer AMA 254 by ALTEC). Cadmium, lead, arsenic, chromium, nickel and zinc were detected by flameless AAS (Varian SpctrAA Zeeman/240) with graphite cell. AAS with flame (AASF) was used to determine copper levels (Varian SpectrAA/600). The heavy metal detection was carried out in an accredited laboratory of State Veterinary and Food Institute in Dolný Kubín. Regarding time restricted hunting season and various legislative conditions, the sampling was not strictly continual and simple process.

Statistical processing of results was carried out by Microsoft Office Excel 2007. Results were expressed as a concentration range ($c_{min.-max.}$), the least square means (average) and median. Tukey's multiple comparison test was used to compare statistical differences among values and $p < 0.05$ was considered a statistically significant difference.[1]

RESULTS AND DISCUSSION

Heavy metal concentration range, average and median in tissue samples (n = 222) of wild living animals from TANAP are shown in Table 2 and Table 3 presents respective data (n = 189) from the Zemplin region. As Lazarus *et al.* (2005) claims, calculated median

1 *min. – max. range of values exceeded the legal limit

values are considered the best heavy metal burden representation among wildlife. Result data from the two monitored localities are presented in Table 4.

- Of the 411 examined samples, in 170 samples the values of the elements were found to be over the permitted limit (41.36 %).
- A significant difference ($p < 0.05$; $t = 0.03162$) in total metal burden between the two localities was found. The more contaminated area due to the presence of contaminants is TANAP. This fact is very interesting because of no direct industrial activities in TANAP area. In TANAP,

Table 2. The heavy metal concentration range, average and median in tissues of wild living animals from Tatra National Park

Animal species		Heavy metal concentration/mg.kg^{-1}					
		Hg	Cd	Pb	As	Cu	Zn
Wild boar	$c_{min.-max}$	0.02-0.871	0.003-2.633	0.009-1.894	0.06-2.94	0.15-53.12	10.90-326.5
	Average	0.147	0.284	0.34	0.586	4.451	59.193
	Median	0.106	0.098	0.286	0.53	1.640	36.50
Red deer	$c_{min.-max}$	N.A.	0.0016-1.80	0.002-1.479	0.006-0.456	N.A.	11.67-101.0
	Average	N.A.	0.289	0.264	0.053	N.A.	29.577
	Median	N.A.	0.127	0.134	0.040	N.A.	27.32
Fox	$c_{min.-max}$	0.013-0.987	0.002-1.24	0.033-13.25	N.A.	1.330-50.0	13.87-142.19
	Average	0.231	0.639	1.137	N.A.	8.210	36.672
	Median	0.15	0.15	4.261	N.A.	2.40	25.24
Roe deer	$c_{min.-max}$	0.005-0.094	0.009-0.508	0.024-1.08	0.001-0.09	0.065-42.80	N.A.
	Average	0.031	0.138	0.159	0.031	13.348	N.A.
	Median	0.02	0.093	0.086	0.021	2.03	N.A.
Wolf	$c_{min.-max}$	0.023-0.68	0.010-0.403	0.090-0.153	0.010-0.368	0.26-22.33	7.31-97.68
	Average	0.042	0.082	0.156	0.081	5.376	30.715
	Median	0.042	0.039	0.154	0.021	4.605	22.46
Chamois	$c_{min.-max}$	0.001-0.019	0.002-1.998	0.006-144.25	0.002-0.010	N.A.	0.002-0.241
	Average	0.004	0.316	11.366	0.005	N.A.	16.982
	Median	0.002	0.016	0.084	0.003	N.A.	16.595
Marten	$c_{min.-max}$	0.025-0.274	0.027-0.557	0.053-0.281	0.02-2.63	2.30-31.25	17.66-67.19
	Average	0.100	0.250	0.195	0.366	7.876	39.426
	Median	0.066	0.191	0.132	0.157	6.343	32.615
Brown bear	$c_{min.-max}$	0.001-0.607	0.002-3.38	0.002-2.26	0.005-0.254	1.06-10.92	5.21-57.15
	Average	0.115	0.510	0.305	0.086	8.506	26.207
	Median	0.075	0.280	0.092	0.038	5.685	22.28
Squirrel	$c_{min.-max}$	0.002-0.161	0.003-0.042	0.008-0.373	0.003-0.794	N.A.	N.A.
	Average	0.082	0.009	0.218	0.573	N.A.	N.A.
	Median	0.085	0.005	0.227	0.571	N.A.	N.A.
Marmot	$c_{min.-max}$	N.A.	0.021-1.36	0.007-0.515	N.A.	0.90-73.43	16.55-164.1
	Average	N.A.	0.247	0.132	N.A.	12.937	43.474
	Median	N.A.	0.124	0.058	N.A.	2.77	34.03
European polecat	$c_{min.-max}$	0.008-0.056	0.005-0.50	0.008-0.442	0.007-1.67	1.18-54.69	23.80-46.87
	Average	0.045	0.17	0.284	0.236	22.915	35.691
	Median	0.055	0.094	0.094	0.062	24,17	37.13
Otter	$c_{min.-max}$	0.02-1.21	0.021-0.358	0.05-0.247	0.03-0.439	7.01-25.68	15.08-66.89
	Average	0.470	0.142	0.161	0.191	11.997	30.991
	Median	0.444	0.100	0.158	0.199	12.08	26.09

N.A. stands for not analyzed

the concentration range of those, which exceeded the limit, was 0.050 – 1.210 mg.kg^{-1}* for Hg, 0.120 – 3.380 mg.kg^{-1} for Cd, 0.101 – 144.25 mg.kg^{-1} for Pb and 0.217 – 2.94 for As. These four are the most frequent heavy metals concentrated in animal organisms there. Nickel is the metal that did not exceeded the limit in TANAP. In Zemplin, the four metals were noticed in the following value ranges respectively: 0.052 – 0.353 mg.kg^{-1}, 0.120 – 6.174 mg.kg^{-1}, 0.107 – 2.414 mg.kg^{-1}, 1.000 – 1.230 mg.kg^{-1}; while there is no value of Cu exceeded the limit.

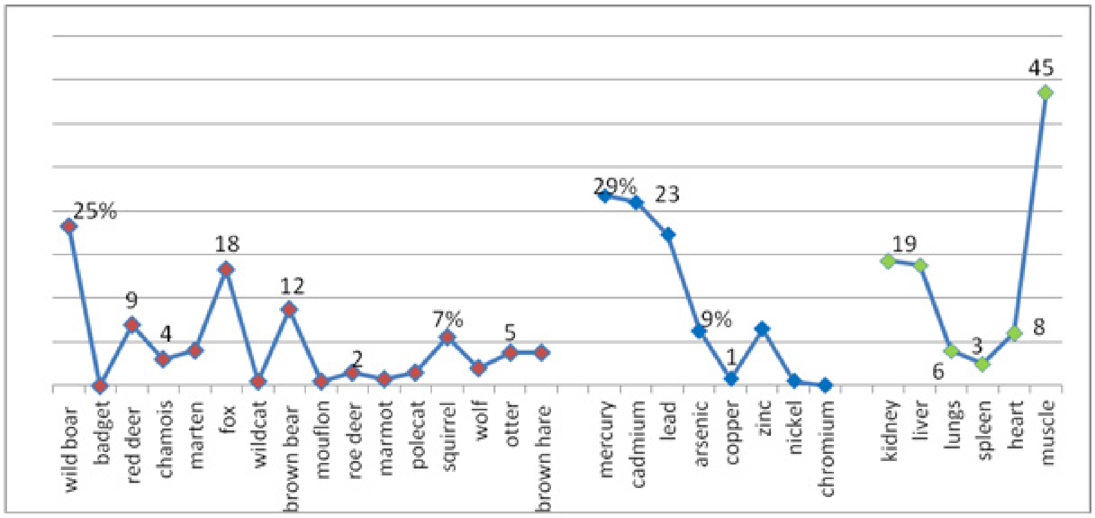
- In TANAP, the highest levels of the four most prevalent metals were detected in following animal species: Hg in otter (median = 0.444 mg.kg^{-1}), Cd in brown bear (median = 0.280 mg.kg^{-1}), Pb in fox (median = 4.261 mg.kg^{-1}) and As in squirrel (median = 0.571 mg.kg^{-1}). In the Zemplin region, it was Hg in wolf, Cd in wild boar, Pb in fox, As in red deer (medians are 0.050; 0.398; 0.279; 0.070 mg.kg^{-1} respectively). We have recorded one special case, when the concentration of Pb reached exceptionally high value (144.25 mg.kg^{-1}) – in chamois (TANAP).
- Generally, the metal most exceeding the legal limits was Hg (0.05 – 1.21 mg.kg^{-1})*, which represents 29 % of all examined samples, then Cd (0.102 – 6.124 mg.kg^{-1}) in 28 %, Pb (0.101 -144.25 mg.kg^{-1}) in 23 %, As (0.217 – 2.94 mg.kg^{-1}) in 9 %, Zn (50.3 – 326.5 mg.kg^{-1}) in 9 %, copper (5.58 – 9.89 mg.kg^{-1}) – 1 %, nickel

Table 3. The heavy metal concentration range, average and median in tissues of wild living animals from Zemplin

Animal species		Heavy metal concentration/mg.kg^{-1}					
		Hg	Cd	Pb	As	Cu	Zn
Wild boar	$c_{min.-max}$	0.001-0.137	0.024-1.53	0.001-0.36	0.001-0.02	0.384-1.294	N.A.
	Average	0.029	0.359	0.188	0.002	0.529	N.A.
	Median	0.002	0.398	0.110	0.001	0.384	N.A.
Red deer	$c_{min.-max}$	N.A.	0.013-0.62	0.002-1.36	0.01-1.23	N.A.	56.17-6.07
	Average	N.A.	0.183	0.304	0.153	N.A.	29.614
	Median	N.A.	0.093	0.095	0.070	N.A.	28.05
Fox	$c_{min.-max}$	0.0015-0.353	0.001-1.11	0.005-1.47	N.A.	N.A.	N.A.
	Average	0.063	0.228	0.296	N.A.	N.A.	N.A.
	Median	0.036	0.031	0.279	N.A.	N.A.	N.A.
Roe deer	$c_{min.-max}$	0.0016-0.09	0.001-0.518	0.003-1.25	0.002-0.823	0.528-42.15	N.A.
	Average	0.024	0.068	0.198	0.164	15.395	N.A.
	Median	0.007	0.009	0.070	0.012	11.380	N.A.
Wolf	$c_{min.-max}$	0.0019-0.82	0.001-0.895	0.005-0.187	N.A.	0.32-5.087	15.64-43.68
	Average	0.140	0.154	0.070	N.A.	4.052	27.848
	Median	0.050	0.028	0.091	N.A.	2.04	21.798
Badger	$c_{min.-max}$	0.0015-0.018	0.001-0.056	0.005-0.091	0.001-0.004	0.17-1.362	N.A.
	Average	0.009	0.024	0.034	0.002	0.839	N.A.
	Median	0,011	0.023	0.026	0.001	1.132	N.A.
Wild cat	$c_{min.-max}$	0.001-0.038	0.002-0.011	0.005-1.47	N.A.	N.A.	N.A.
	Average	0.004	0.005	0.145	N.A.	N.A.	N.A.
	Median	0.001	0.004	0.041	N.A.	N.A.	N.A.
Mouflon	$c_{min.-max}$	0.0015-0.83	0.01-0.518	0.014-1.045	N.A.	N.A.	N.A.
	Average	0.036	0.130	0.258	N.A.	N.A.	N.A.
	Median	0.026	0.074	0.078	N.A.	N.A.	N.A.
Hare	$c_{min.-max}$	0.001-0.075	0.001-6.174	0.004-2.414	0.0001-1.0	0.80-4.806	N.A.
	Average	0.021	0.387	0.221	0.041	2.473	N.A.
	Median	0.002	0.032	0.064	0.030	2.283	N.A.

N.A. stands for not analyzed

Table 4. The percentage data of the results from the two monitored localities



(0.872 – 1.083 mg.kg^{-1}) in 1 % of the samples. No value exceeded the limit was recorded in chromium.

- In total, the most contaminated animal species (the most data exceeded the legal limit) was wild boar (25 %), then fox (18 %), brown bear (12 %), red deer (9 %), squirrel (7 %), otter (5 %), brown hare (5 %), marten (5 %), chamois (4 %), wolf (3 %), roe deer (2 %), polecat (2 %), marmot (1 %), wildcat (1 %), mouflon (1 %), badger (0 %). The most heavy metal burdened animal species in TANAP was wild boar, then brown bear, fox and squirrel. In Zemplin it was fox, hare, red deer and wild boar. Wild boar was the species with the most significant heavy metal levels in both localities.
- The most attacked organ is the muscle (45 %), liver and kidney (19 %), heart (8 %), lungs (6 %) and the least affected is spleen (3 %).
- From the analysis of the elements in 40 biotope constituents taken from TANAP, the maximum permissible values exceed all three determined elements (Hg, Cd, Pb). The highest over-limit for Hg was measured in the sample of moss (0.893 mg.kg^{-1}).The highest supernatant Cd was in the sample of bark (2.120 mg.kg^{-1}), the lowest in the grass sample (0.082 mg.kg^{-1}). Pb in leaf litter was exceded by more than 85.20 mg.kg^{-1}. The contamination of the constituents was in the following descending order: Moss – Bark – Grass – Leaf litter – Needles (Figure 2).

Several authors (Mauro F *et al.*, 2017) studied the heavy metal environmental burden by heavy metals. In Slovakia, Kováč *et al.* (2005) detected concentrations of Cd, As, and Pb in body tissues of wild living red deer and wild boar game that fell within the legal limit value range (Food Codex, Slovak Republic). However, the authors show differences between individual game species which is a consequence of their distinct way of life, food intake and composition. According to our results, variability in animal species contamination because of different life conditions and feeding manners was also confirmed. Wild boar was the species with significant heavy metal levels in both localities. We suspect wild boar game as typical omnivores to be more exposed to environmental burden. The higher potential risk of food contamination by metals and subsequent metal accumulation in body tissues impend. Between the year 2001 and 2003 concentration values of Pb that exceeded the legal limit (according the Food Codex) in the family Cervidae were recorded in 2.6 % of the examined samples (Šalgovičová and Krížová, 2004). Nowadays, lead has the third position in risk ladder of heavy metal contamination (after Hg and Cd). In our study, Pb exceeded limit in 23 % of the samples what is significantly higher value. Especially two individual cases were significant – in chamois (144.25 kg.mg^{-1}) and wild boar (2.414 mg.kg^{-1}) from TANAP, in fox from the Zemplin region. Bilandžić *et al.* (2010) also confirm significant heavy metal (Cd, Pb,Hg) contamination in wild boar in Croatia.

In this study, mercury and cadmium were recorded as the most prevalent in the monitored localities. Other authors also present mercury and cadmium as exceeding the limit levels in various kinds of wild living animals (Kramárová *et al.,* 2005; Piskorová *et al.* 2003; Pompe-Gotal *et al.*, 2009). Contrary to our results, Piskorová *et al.*, (2003) detected chromium as over-limited in 6.6 % of samples. Our research has not revealed dangerous level of chromium. In eastern Croatia, red deer was examined for heavy metal levels in tissues (Lazarus *et al.* 2005). The median concentration of toxic cadmium, mercury, and lead in the kidney were 0.099 mg.kg^{-1}, 0.362 mg.kg^{-1}, and 0.578 mg.kg^{-1}, respectively. In the jawbone, the Pb mass fraction was 0.281 mg.kg^{-1}. In comparison, our study provides median values of Cd and Pb concentration in red deer respectively: 0.127 mg.kg^{-1} and 0.134 mg.kg^{-1} in TANAP; 0.093 mg.kg^{-1} and 0.095 mg.kg^{-1} in Zemplin. Mercury was not detected in red deer.

Many authors describe the metal concentration in various types of body tissues (e.g. Andreotti *et al.*, 2016; Bellinger *et al.*, 2013; Bernhoft *et al.*, 2014; Hunt *et al.*, 2009; Juric *et al.*, 2018; Knott *et al.*, 2010). The muscle, liver, kidney and fat samples of 20 roe deer of both sexes originating from a hunting area in central Hungary were investigated by Lehel *et al.* (2017) for the presence of heavy metals such as As, Cd, Hg and Pb, and their contents were evaluated for possible health risk to consumers. Based on the data obtained from the present study, the consumption of organs and tissues of the investigated roe deer could be objectionable from food-toxicological point of view and may pose risk to the high consumers of wild game due to their cadmium and lead contents.

In this study, we did not deal with the aspect of metal distribution into individual tissues because of not-equable sample amount. Most of our tissue samples come from hares. Cadmium and lead were the metals, the concentrations of which represented the highest levels in this game. Levels of cadmium, lead and mercury in hare tissues were also examined in south-western Slovakia (Slamečka *et al.*, 1994). Levels of Hg and Cd are significantly increased in body tissues (liver, kidneys), depending on the increasing age of the hares.

During the period from 2002 to 2004 fifteen individuals of brown bear from Carpathians were examined to detect heavy metal levels and their distribution into the body tissues (Čelechovská *et al.,* 2006). The highest concentrations of Cd, Pb, Hg were recorded in kidneys (17.4 ± 5.2 mg.kg^{-1}, 1.16 ± 0.39 mg.kg^{-1}, 0.39 ± 0.25 mg.kg^{-1}). During our study we managed to gain 26 samples of brown bear from the TANAP locality. The metals exceeding

Figure 2. Samples of biotope components (leaf litter, needles, moss, grass)

the legal limit the most were Cd (13 samples), Hg (9 samples), Pb (6 samples), Cu (5 samples), As (3 samples), Zn (1 sample).

In order to detect environmental contamination, vegetation could be used as proper material for laboratory testing. Just as in our study, Bykowszczenko *et al.* (2006) discovered environmental burden with heavy metals in a national park in Poland detecting the content in mosses. Słowiński National Park is also a protected area in the central part of the Polish Baltic coast. Contrary to our results, the Polish research suggest a reduction of heavy metal contamination in this national park over the last 27 years and confirmed that the area is one of the cleanest in Poland and may still serve as a reference background for determining pollution in other areas. Słowiński National Park is under relatively small threat from gas and dust pollution compared with the other national parks in Poland. This is due to its location in a lightly inhabited area of the Baltic coast, far from the industrial centres. Despite the industrial emissions and dust from long-distance transport still present in the area, the natural environment of Słowiński National Park is relatively unaffected. Kozanecka *et al.* (2002) also monitored the heavy metal contamination of pollution-free regions. The stated concentration of Zn, Cu, Pb, Ni, Cr and Cd were very little differentiated considering particular plant species of forest floor. And those were appreciated at the natural level, typical for the unpolluted area. Many substances accumulated in animal and human organisms are not needed for physiological activities. On the contrary, these substances can cause various pathological changes and serious health disorders. Accumulating in body tissues, content of the materials usually increase with aging, thus they start acting as toxic. This is related to e.g. arsenic, cadmium, lead, mercury, etc. (Toman *et al.*, 2003a). The main way of receiving metals into living organisms is food intake, so the monitoring in food-stuff of human and animals is essential (Golian *et al.*, 2004). Grazing animals receive heavy metals from contaminated soil that can create 18 % of total ingested dry mass in cattle and 30 % in sheep. The quickest way for metal absorption is in the middle part of small intestine. Absorbed metals bind to blood cells or blood plasma components (Gallo, 1995). Even the type of grazing can affect the heavy metal levels in the environment. Results of Majid Ajorlo *et al.* (2010) suggest that the excreta of grazing cattle can be an important source of heavy metals in intensively managed pastures in the long-term. However, the metal concentrations were maintained within the normal range and were not high enough to be dangerous from the toxicological point of view.

Research of wild living animals is difficult and very important. It brings a lot of knowledge about the ecological stability changes in select forest localities that can be applied in practice (Begon *et al.*, 1997). In Slovakia, the actual state of forests is disordered by emission and other pollutant effect.

CONCLUSIONS

Generally, physical environmental pollution (air, water, soil) in Slovakia has different tendency in various regions due to new conditions of the market economy that results from permanent changes in the assortment processing. Heavy metal cumulation in ecosystems is a serious problem of environment quality. It is known that the danger of environmental pollution is still impending because of the permanent negative effects of the industrialised country (exhalation, soil contamination, surface and phreatic water contamination, etc.) and motorization. Regarding the life cycle of wild living animals we can suppose that wild animals permanently exposed to external environment have higher levels of metal contamination in comparison to domestic animal breeding in internal stables (Niemi *et al.*, 1993; Kugonič and Zupan, 1999; Košutzký *et al.*, 2003; Krížová and Šalgovičová, 2001). Besides trophy quality production, wild living animals are a suitable bioindicator of biotope quality, as well. Thus it is convenient to use this fact to take care of human life quality (Tataruch, 1995; Krynski *et al.*, 2003; Zmetáková and Šalgovičová, 2008). Animal indicators also help in detecting the amount of toxins present in the tissues of animals (Joanna, 2006; Khatri and Tyagi, 2015).

The results of this study confirm heavy metals contamination even in game that comes from an area preserved and restricted in any industrial activity – Tatra National Park. As presented, reduction or total elimination of the industrial use of an area do not strictly mean elimination of environmental burden there. We suppose that the environment contamination and higher pollutant burden in TANAP is a consequence of Katowice industrial complex in Poland (Degórska, 2013). Allegedly dominant north air flow supports emission transport from heavy industry to this relatively clean and virgin area (Makovníková and Kanianska, 2003). The results can serve as basic data for next ecological and veterinary study in Slovakia. It is necessary to continue in further parameter collection with subsequent detection of pollutants in other material samples, e.g. in vegetation (moss, conifer needles, etc.) which is another proper bioindicator of environmental contamination. In order to eliminate the negative effect of contaminants on human and animal population in the monitored areas as well as on the whole territory of Slovakia, it is essential: to investigate risk factors in the environment (to know the contamination situation), to decrease production of the metallurgical industry emissions, to reduce agricultural area contamination by restricted pollutant entry through soil, to eliminate the metal absorption in animals (antidote administration, adaptation of food composition), to use legislative and economic means for forcing the polluter to take responsibility for environment pollution losses and costs, to educate the human population in environmental management.

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