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The effect of heavy metals in the water source on selected biochemical indices in European mouflon (*Ovis musimon* L.) from a game reserve

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Abstract

The aim of our study was to analyse the presence of selected heavy metals in surface water located in a game reserve, and their effect on selected biochemical indices in female mouflon. The results of statistical testing of the experimental group of female mouflon in 2014 and 2015 confirmed a significant negative dependency ($P < 0.05$) between albumin and aspartate aminotransferase and selected heavy metals. A significantly negative correlation ($P < 0.01$) was shown between phosphorus and creatinine and selected heavy metals. In case of bilirubin and urea, we observed a significant positive correlation with selected heavy metals ($P < 0.05$). A significantly negative correlation ($P < 0.0001$) was observed between calcium and selected heavy metals. Our results point out the interaction of selected heavy metals and selected biochemical indices.

Biomarker, environment, female, herd, wildlife

The concentration and mobility of heavy metals in the environment has radically increased in the last 100 years. Their increased concentration in the food chain can cause significant health consequences in the long term (Heroldova et al. 2007). Toxic metals bind to the functional groups of bio molecules, changing their structure and function, and act as enzyme toxins (Jezný et al. 2014). The impact of heavy metals on various biochemical markers is considerable due to a lifetime exposure. Disease can cause substantial costs for the game farmer and results in serious problems regarding animal welfare and food safety (Beníšek et al. 2000). Biochemical markers can be used as a tool for monitoring the game herd's health status (Žele and Vengušt 2012). The aim of our study was to analyse the presence of selected heavy metals in surface water located in the present game reserve and their effect on selected biochemical indices in female mouflon.

Materials and Methods

The mouflon used in the research came from a game reserve in the Eastern part of the Slovak Republic. Water intake was unrestricted from natural water sources. The mouflon were fed hay during the winter time. They showed no signs of disease. Blood was taken from the jugular vein by a veterinarian. The experimental group consisted of females (n = 15), at approximately the same age and average live weight of 34.5 kg. Samples were collected in spring using blood collection tubes (Serum Separator Tubes -SST™ II Advance and Plasma Separation Tubes – PST™ II containing lithium heparin and gel, BD Diagnostics, USA) and immediately centrifuged. Blood samples were taken and analysed in years 2014 and 2015. Serum was stored at -20 °C until analysed in case

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of serum for the results for LDL were analysed immediately after centrifugation. The following biochemical indices were analysed: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), glucose (GLU), bilirubin (BIL), calcium (Ca), phosphorus (P), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TRIG), creatinine (CREA), and urea (UREA). Biochemical indicators were measured using the automatic analyser COBAS® c111, with a flexible system for consolidating routine examinations, according to manufacturer's guidelines (Roche Diagnostics, Switzerland). Water samples for the analysis of presence of selected heavy metals were collected from three sampling places in the game reserve. Water sampling was analysed by microprocessor controlled colorimeter, Hach Dr/890 suitable for colorimetric detection (Hach, USA). Of the heavy metals present in water sources, manganese (Mn), copper (Cu), lead (Pb), zinc (Zn), and iron (Fe) were analysed. The biochemical indices were evaluated in relation to the presence heavy metals in water sources. The obtained results were statistically analysed by Mann-Whitney's U test for comparisons of biochemical indices between females depending on the year and the biochemical properties. Spearman's correlation coefficient was used for identify correlation between selected biochemical indices of females and the mean content of heavy metals in the years.

Results

Analysis of water samples confirmed the presence of selected heavy metals in spring of years 2014 and 2015 from three surface resources within the game reserve. Figs 1 and 2 report the mean values of selected heavy metals Fe, Mn, Cu, Pb, and Zn in water sources in 2014 and 2015.

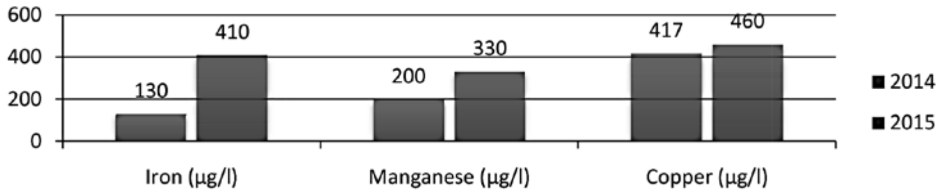

Fig. 1. Mean values of selected heavy metals in the water sources analysed in the years 2014 and 2015

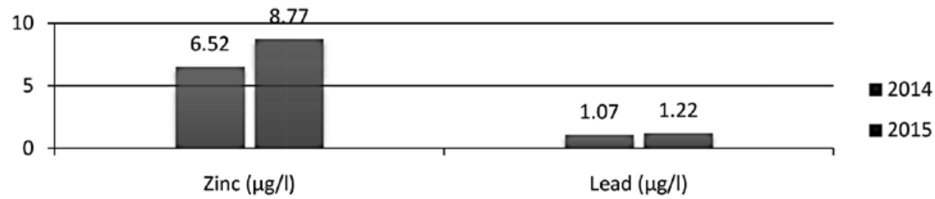

Fig. 2. Mean values of selected heavy metals in the water sources analysed in the years 2014 and 2015

The mean increase in total iron was almost threefold in 2015 compared to 2014. Compared to 2014, the increase of the mean values of selected heavy metals in water sources in 2015 was 65% for manganese, 10.31% for copper, 14.02% for lead and 34.51% for zinc. Comparison of the mean values of selected heavy metals by years found an overall increase of the heavy metals in 2015 compared to 2014. The mean values of selected biochemical indices in 2014 and 2015 are presented in Tables 1 and 2.

For comparison of various biochemical indices of females in 2014 and 2015 we used Mann-Whitney's U test with a significance level of $P < 0.05$. Significant differences were found for ALP ($P = 0.3165$), ALT ($P = 0.2535$), and LDH ($P = 0.3391$) comparing

Table 1. The mean values of selected biochemical indices in the year 2014.

n = 15	GLU (mmol/l)	ALB (g/l)	ALP (ukat/l)	ALT (ukat/l)	AST (ukat/l)	BIL (umol/l)	Ca (mmol/l)	CHOL (mmol/l)	CREA (umol/l)	HDL (mmol/l)	LDH (U/l)	LDL (mmol/l)	P (mmol/l)	TRIG (mmol/l)	UREA (mmol/l)
x	2.631	10.865	0.457	0.299	1.075	0.409	1.982	0.597	55.987	0.475	380.46	0.155	1.047	0.341	3.531
± std	0.895	3.086	0.122	0.125	0.268	0.241	0.331	0.288	8.701	0.210	129.8	0.082	0.259	0.190	1.956
Min	1.09	7.94	0.28	0.117	0.688	0.11	1.32	0.28	45.9	0.212	221	0.06	0.64	0.14	1.47
Max	4.48	17.07	0.72	0.456	1.581	0.74	2.64	1.1	81.79	0.763	610	0.33	1.46	0.77	6.9
Median	2.51	9.42	0.42	0.304	0.996	0.36	2.09	0.47	53.97	0.419	365	0.14	1.09	0.3	2.85

n – number of singulars, x – mean, min – minimum, max – maximum, GLU – glucose, ALB – albumin, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, BIL – bilirubin, Ca – calcium, CHOL – cholesterol, CREA – creatinine, HDL – high-density lipoprotein, LDH – lactate dehydrogenase, LDL – low-density lipoprotein, P – phosphorus, TRIG – triglycerides, UREA – urea, Fe – iron, Mn – manganese, Cu – copper, Pb – lead, Zn – zinc.

Table 2. The mean values of selected biochemical indices in the year 2015.

n = 15	GLU (mmol/l)	ALB (g/l)	ALP (ukat/l)	ALT (ukat/l)	AST (ukat/l)	BIL (umol/l)	Ca (mmol/l)	CHOL (mmol/l)	CREA (umol/l)	HDL (mmol/l)	LDH (U/l)	LDL (mmol/l)	P (mmol/l)	TRIG (mmol/l)	UREA (mmol/l)
x	3.266	8.990	0.429	0.270	0.805	0.708	1.053	0.598	38.679	0.465	374.867	0.115	0.661	0.219	4.885
± std	1.202	3.933	0.230	0.153	0.264	0.263	0.188	0.364	14.065	0.259	170.03	0.073	0.357	0.122	0.889
Min	0.78	4.73	0.1	0.098	0.438	0.39	0.65	0.19	22.77	0.151	163	0.04	0.16	0.06	3.22
Max	6	17.13	0.73	0.657	1.266	1.35	1.29	1.41	64.02	1.013	714	0.27	1.27	0.44	6.69
Median	3.47	7.77	0.39	0.266	0.724	0.59	1.1	0.41	34.78	0.338	303	0.09	0.59	0.19	4.84

n – number of singulars, x – means, min – minimum, max – maximum, GLU – glucose, ALB – albumin, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, BIL – bilirubin, Ca – calcium, CHOL – cholesterol, CREA – creatinine, HDL - high-density lipoprotein, LDH – lactate dehydrogenase, LDL - low-density lipoprotein, P – phosphorus, TRIG – triglycerides, UREA – urea, Fe – iron, Mn – manganese, Cu – copper, Pb – lead, Zn – zinc.

the years 2014 and 2015. Comparing the values of AST in 2014 and 2015, significant differences (P = 0.0081) were found. No significant differences were found for CHOL (P = 0.4752) and HDL (P = 0.4752) comparing the years 2014 and 2015. The measured values of LDL in 2014 were found significantly different (P = 0.0462) compared to the values of LDL in 2015. Significant differences in TRIG were also observed (P = 0.031) comparing the years 2014 and 2015. Significant differences were found for

Table 3. Correlation dependence between selected biochemical indices of females and the mean content of heavy metals in the years 2014 a 2015.

	Females 2014, 2015				
n = 30	Fe	Mn	Cu	Pb	Zn
GLU	ns	ns	ns	ns	ns
ALB	-0.381263*	-0.381263*	-0.381263*	-0.381263*	-0.381263*
ALP	ns	ns	ns	ns	ns
ALT	ns	ns	ns	ns	ns
AST	-0.450734*	-0.450734*	-0.450734*	-0.450734*	-0.450734*
BIL	0.439226*	0.439226*	0.439226*	0.439226*	0.439226*
Ca	-0.866893 ***	-0.866893 ***	-0.866893 ***	-0.866893 ***	-0.866893 ***
CHOL	ns	ns	ns	ns	ns
CREA	-0.550713**	-0.550713**	-0.550713**	-0.550713**	-0.550713**
HDL	ns	ns	ns	ns	ns
LDH	ns	ns	ns	ns	ns
LDL	ns	ns	ns	ns	ns
P	-0.527900**	-0.527900**	-0.527900**	-0.527900**	-0.527900**
TRIG	ns	ns	ns	ns	ns
UREA	0.388965*	0.388965*	0.388965*	0.388965*	0.388965*

n – number of singulars, ns – not significant, *** – correlation is significant at the level of 0.0001, ** – correlation is significant at the level of 0.01, * – correlation is significant at the level of0.05, GLU – glucose, ALB – albumin, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, BIL – bilirubin, Ca – calcium, CHOL – cholesterol, CREA – creatinine, HDL – high-density lipoprotein, LDH – lactate dehydrogenase, LDL – low-density lipoprotein, P – phosphorus, TRIG – triglycerides, UREA – urea, Fe – iron, Mn – manganese, Cu – copper, Pb – lead, Zn – zinc.

Ca ($P < 0.0001$), P ($P = 0.0024$), GLU ($P = 0.0373$), ALB ($P = 0.0211$), BIL ($P = 0.0095$), CREA ($P = 0.0016$) and UREA ($P = 0.019$) comparing the years 2014 and 2015.

Table 3 shows correlations between selected biochemical indices of female mouflon and the mean content of heavy metals in the years 2014 and 2015.

The result obtained in 2014 and 2015 confirmed a significant negative dependency ($P < 0.05$) between ALB and AST and the selected heavy metals. Significant negative dependency ($P < 0.01$) was shown between P and CREA and the selected heavy metals. In case of BIL and UREA we recorded a significant positive correlation with the selected heavy metals ($P < 0.05$). Significant negative correlation ($P < 0.0001$) was shown between Ca and the selected heavy metals.

Discussion

Surface water in the Slovak Republic is considered mainly as a sensitive area of deterioration of watercourses which represents an increased risk for many kinds of organisms and their health status. At present, no similar research was found in Slovakia, evaluating the impact of heavy metals in the drinking sources and their interaction with biochemical properties. The impact of heavy metals in water sources on the biochemical properties of animals is studied in detail worldwide, as confirmed by several foreign studies. Abd El-Rahman et al. (2005) evaluated the effect of heavy metals in water sources and their toxicity on the biochemical status of goats and sheep, and their results correspond with ours. Analysis of wild animal biochemical indices undertaken by the Peinado et al. (1999) reported higher values of TRIG (0.83 mmol/l) and UREA (8.87 mmol/l) compared to our measured value of TRIG (0.341 mmol/l) and UREA (3.531 mmol/l) in 2014. The value of BIL

0.409 µmol/l was lower than the BIL 4.6 µmol/l reported by the compared authors. Other authors (Mostaghni et al. 2005) examined the haematological and biochemical indices in wild sheep reared in Iran. The values of GLU 3.266 mmol/l, ALB 8.990 g/l, BIL 0.708 µmol/l, CREA 38.679 µmol/l and LDH 374.867 U/l that we measured in females in 2015 were lower as mean values than those of the compared authors who found the mean values of GLU 7.64 mmol/l,ALB 34 g/l, BIL 1.88 µmol/l, CREA 173.87 µmol/l and LDH 1484.14 U/l. Haematological and biochemical statuses in mouflon were analysed in Southern Europe by Marco et al. (1998) who recorded higher mean values of CHOL 0.95 mmol/l, TRIG 0.39 mmol/l, Ca 2.27 mmol/l, P 2.35 mmol/l, GLU 3.69 mmol/l, ALB 26.3 g/l, BIL 6.67 µmol/l, and CREA 84.0 µmol/l compared to the values of CHOL 0.598 mmol/l, TRIG 0.219 mmol/l, Ca 1.053 mmol/l, P 0.661 mmol/l, GLU 3.266 mmol/l, ALB 8.990 g/l, BIL 0.708 µmol/l, and CREA 38.679 µmol/l we found in our experimental group of animals in 2015. Crivineanu et al. (2010) investigated the correlation between selected heavy metals in the environment and their concentration in the blood of sheep reared on a farm in the province of Thessalonica. They found the presence of heavy metals at concentrations of Zn 92.7 µg/l, Fe 21 µg/l, Pb 2.05 µg/l in water sources and also confirmed the presence of selected heavy metals in blood of animals that drank from this water source.

As this was the first study in a mouflon herd, no suitable studies were found for relevant comparison. Due to the lack of research on this topic, we aimed to highlight the interaction between selected heavy metals (i.e. iron, manganese, lead, copper, and zinc) and selected analysed biochemical indices in female mouflon. The results showed that the heavy metals were negatively correlated with albumin, aspartate aminotransferase, calcium, creatinine, and phosphorus, and positively associated with bilirubin and urea.

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