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THE INCIDENCE OF HEAVY METALS AND OTHER TOXIC ELEMENTS IN ROE DEER (*Capreolus capreolus)* TISSUES

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Abstract

Levels of lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg) and copper (Cu) in the liver, kidney and muscle of 11 individual roe deer (*Capreolus capreolus)* were determined. The samples were prepared by microwave wet digestion. Content of investigated elements was determined by the method of coupled plasma with mass spectometry. The lead concentrations ranged from <0.001 (liver) to 8.455 mg/kg (meat), Cd concentrations ranged from 0.004 (muscle) to 0.818 mg/kg (kidney) and As concentrations ranged from 0.002 (liver) to 0.031 mg/kg (kidney). Concentrations of Hg in examined tissues (liver, kidney, muscle) were under limit of detection (<0.001 mg/kg). The concentration of copper in liver ranged from 3.913 to 104.08 mg/kg. The results of this study showed that no samples exceeded maximum allowed levels for Cd, Hg, As and Cu. Pb concentrations in muscle samples ranged from 0.008 to 8.455 mg/kg. High concentrations of Pb in two muscle samples are most likely due to the proximity of hunting wound area, as lead was not detected in organ samples. The presence of some elements in the tissues of roe deer suggests the necessity of further research aimed at identifying the source of contamination in order to preserve the health of both humans and animals.

Keywords*:* lead, cadmium, mercury, arsenic, copper, tissues, roe deer

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TEŠKI METALI I DRUGI TOKSIČNI ELEMENTI U TKIVIMA SRNA (*Capreolus capreolus)*

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Kratak sadržaj

U ovom radu ispitivan je sadržaj olova (Pb), kadmijuma (Cd), arsena (As), žive (Hg) i bakra (Cu) u jetri, bubrezima i mišićnom tkivu, 11 srna. Uzorci su pripremljeni metodom mikrotalasne vlažne digestije. Sadržaj ispitivanih elemenata je određen metodom indukovano kuplovane plazme sa masenom detekcijom. Koncentracija Pb kretala se u intervalu od <0,001 (jetra) do 8,455 mg/kg (mišićno tkivo), dok se koncentracija Cd kretala od 0,004 (mišićno tkivo) do 0,818 mg/kg (bubreg). Koncentracija As je bila u rasponu od 0,002 (jetra) do 0,031 mg/kg (bubreg). Koncentracije Hg u ispitivanih tkivima (jetra, bubreg, mišić) je bila ispod granica detekcije metode (<0,001 mg/kg). Sadržaj bakra u jetri kretao se u intervalu od 3,913 do 104,08 mg/kg. Izmerene vrednosti za sadržaj Cd, Hg, As i Cu u svim ispitivanim tkivima, a sadržaj Pb u jetri i bubrezima ne prelazi propisane maksimalno dozvoljene vrednosti. Koncentracije Pb u uzorcima mišićnog tkiva kretala se u rasponu od 0,008 do 8,455 mg/kg. Visoke koncentracije Pb u dva uzorka mišića, verovatno su posledica blizine mesta odstrelne rane, s obzirom na to da u uzorcima organa istih životinja nije detektovano Pb. Zbog prisustva toksičnih elementa u tkivima i organima srna, neophodna su dalja istraživanje kako bi se identifikovali izvori kontaminacije, a u cilju očuvanja zdravlja ljudi i životinja.
Ključne reči: olovo, kadmijum, živa, arsen, bakar, tkiva, srna

INTRODUCTION

Contamination of the environment with hazardous compounds and elements of anthropogenic origin is of increasing concern because of its effect on the entire biosphere, i.e., the micro-flora and –fauna of soils, plants and higher life, including humans and animals (Selenius et al., 1996). Many wild animals are exposed to diverse toxic substances by consuming contaminated plants and animals, or water, soil and air (Živkov Baloš et al., 2015). The accumulation of toxic heavy metals in plants and soil may increase the risk of transfer to

herbivorous wild mammals and game animals or to livestock (Bilandžić et al., 2010). As animals can move freely and find their own food, the game is a link in the chain that accumulates pollutants from the environment. Monitors have recently been defined as organisms in which changes in known characteristics can be measured to assess the extent of environmental contamination, so that conclusions on the health implication for other species of the environment as a whole can be drawn. Monitors may provide information about environmental concentrations of essential and toxic metals of importance for life, displaying deficiency and toxicity, respectively (Selinus et al., 1996).

In order to fully understand the exposure of animals to many pollutants originating from the environment and to assess the harmful effect and estimate the risk, it is necessary to carry out a systematic study and gather data on degree and type of pollution, as well as distribution of hazardous chemicals in nature. Nowadays, a number of studies have been based on the determination of chemical contaminants in animal tissues and organs. As the result of these findings, it is possible to estimate the level of human exposure to negative effects of these pollutants. The monitoring and control of game meat safety should include control measures for live animals, control measures during hunting and after shooting, guidelines for official meat inspection, control measures for carcass processing and surveillance of chemical residues (Petrović et al., 2014).

Since it fulfils numerous criteria (e.g. widely geographic distribution, relatively small home range, territorial living and browsing nutrition strategy, huge availability of basic data, relatively simple sampling procedure) roe deer has been often mentioned in the literature as a good or even excellent monitor of toxic elements burdens on the environment (Pokorny, 2000).

The purpose of this study was to evaluate the concentration of environmental contaminants lead (Pb), arsenic (As), mercury (Hg), cadmium (Cd) and copper (Cu) in tissues of free-living roe deer in Serbia, as important information in performing assessments of the risk for both wildlife and humans. The obtained results were compared with relevant data reported from other countries.

MATERIAL AND METHODS

Samples of liver, kidney and meat of roe deer *(Capreolus capreolus)* shot by hunters were collected from hunting ground of Begeč settlement, municipality Novi Sad. Sampling of wild animal organs was performed during the 2013/2014 hunting season. Animals were selected according to neither sex nor age. Thus, liver and kidney samples were collected from each animal (total of

11 animals). Upon collection, all samples were placed into labeled plastic bags and stored at -18° C to avoid tissue degradation prior to analysis.

The samples (1g) were prepared applying the microwave (Ethos, Labstation Microwave, Milestone), digestion method (14) with the use of the mixture H_2O_2/HNO_3 (1:4, v/v). After this process, the samples were transferred to 50 mL volumetric flasks and diluted with deionized water. Analyses of Pb (NoG-M, IT 0.1 s/P), Cd (NoG-M, IT 1 s/P), As (He-M, IT 1 s/P), Hg (NoG-M, IT 1 s/P) and Cu (He-M, IT 0.1 s/P) were conducted by ICP-MS 7700 mass spectrometer (Agilent Technologies). Solutions used for calibration were prepared from commercial stock standard solutions with 1000 mg/l of each element (Accustandard). To calculate the recovery percentage, 6 samples of meat have been spiked with known amounts of Cd, As, Hg, Pb and Cu analytical standards. The obtained results are presented in Table 1.

Table 1: Isotopes, limit of detection (LOD) and recovery rates for monitored elements

Element	Isotope	LOD (mg/kg)	Recovery (%)
Cd	^{111}Cd	0.001	96.1
As	^{75}As	0.001	100.4
Hg	^{201}Hg	0.001	83.7
Pb	^{208}Pb	0.001	88.1
Cu	^{63}Cu	0.001	102.9

Statistical analysis was performed by the STATISTICA 12 software package, version 16.0. Data were grouped according to tissue and presented as mean ± standard error, minimum and maximum values.

RESULTS AND DISCUSSION

Average values of toxic and trace elements obtained in this study for the livers, kidneys and meat of all investigated roe deer are summarized in Table 2. The obtained values were compared with highest permissible hygienic limits for risk elements according to the maximum allowed levels (MAL) of particular contaminant in food in the Republic of Serbia (Official Gazette, 2011).

The results were compared with the results reported by other authors from our and other countries. Beside that comparison, a collation with some other countries could be interesting as well. An overview of some previous articles addressing toxic elements levels in tissues of free living roe deer *(Capreolus*

capreolus) and red deer (*Cervus elaphus)* is presented in Table 3.

Table 2. Toxic elements concentrations (mg/kg) in different tissues of roe deer

Material	n	**Cd**	**As**	**Hg**	**Pb**	**Cu**
		Mean±S.E. *Range*				
Liver	11	0.0527 ± 0.043 0.005-0.110	0.008±0.006 *0.002-0.017*	< 0.001	0.077 ± 0.092 *<0.001-0.222*	28.071±34.026 *3.913 -104.08*
Kidney	11	0.465±0.224 *0.166-0.818*	0.014±0.008 *0.005-0.031*	<.0.001	0.094±0.107 *<0.001-0.2900*	15.871±6.698 *0.6890 -28.390*
Muscle	4	0.005±0.008 *0.004-0.008*	0.014 ± 0.004 *0.01-0.02*	<.0.001	*0.008-**8.455***	4.2305±0.9411 *3.393-5.568*
MAL	mg/kg	liver 0.50 kidney 1.0 meat 0.05	liver 0.50 kidney 0.50 meat 0.10	liver 0.10 kidney 0.10 meat 0..03	liver 0.50 kidney 0.50 meat 0.10	liver 80.0

MAL -Maximum allowed level (Official Gazette, Republic of Serbia, 2011)

Concentrations of As, Cd and Hg in liver, kidney and muscle samples of roe deer did not exceed the MAL in either of the examined samples (Table 2).

The highest Cd-contamination (average value 0.465 mg/kg) was recorded in the kidneys of roe deer. Somewhat lower Cd levels were found in the liver (0.0527 mg/kg), while muscles were the least contaminated (0.005 mg/kg). The lowest and highest average concentrations of As were measured in liver (average value 0.008 mg/kg) and kidney samples (0.031 mg/kg), respectively. Mercury (Hg) was not detected in any of the investigated samples. Kidneys of roe deer revealed higher Pb-contamination than liver (average values 0.094 mg/kg in kidney and 0.077 mg/kg in liver). The Pb content in two muscle samples of roe deer was very high. High Pb concentrations measured in muscle samples are probably due to the proximity of gunshot wound, especially since such enormously high level of Pb in the examined muscle sample *show* a *strong discrepancy with* respect to Pb values in the liver and kidney (lead was not detected in samples of these organs). The maximum permissible level for Cu is prescribed only for liver (80 mg/kg). The average Cu level detected in the liver was 28.071±34.026 mg/kg, while the value exceeding MAL (104.08 mg/kg) was recorded in only one sample.

Table 3. Content of toxic elements (mg/kg) in different tissues of roe deer and red deer according to various authors

Material	As	Cd	Hg	Pb	Cu	Source, species and country
	Mean±S.E. Range					
Liver	n.i.	0.568±0.502 0.015-2.306	n.i.	n.i.	n.i.	Pompe –Gotal J. and Prevendar Crnić A. (2002) Capreolus capreolus Croatia
Kidney		4.905±6.395 0.223-27.686				
Muscle		0.018±0.019 0.003-0.065				
Liver	n.i.	1.06±0.77 (yearlings) 3.92±0.88 (2 year and more)	n.i.	0.71±0.65 <0.05-9.3	n.i.	Pokorny B. and Ribarič-Lasnik C. (2000) Capreolus capreolus Slovenia
Kidney		7.13±4.43 (yearlings) 22.73±8.92 (2 year and more)		0.03±0.01 <0.05-0.20		
Muscle		0.03±0.02 (yearlings) 0.04±0.01 (2 year and more)		0.05±0.03 <0.05-0.55		
Liver	n.i.	0.21±0.10	n.i.	1.40±0.01	n.i.	Kottferová J. and Koréneková B. (1998) Capreolus capreolus Slovakia
Kidney		2.63±2.24		0.25±0.18		
Muscle		0.02±0.03		0.12±0.03		
Liver	n.i.	0.70±0.39*	n.i.	0.17±0.11*	59±41*	Jarzyńsla G. and Falandysz J. (2011) Cervus elaphus Poland
Kidney		12±8 *		0.30±0.26*	21±4*	
Muscle		0.22±0.13*		0.18±0.34*	11±4*	
Kidney	n.i.	2.071±0.216 0.010-22.076	n.i.	n.i.	n.i.	Beiglböck C. et al. (2002) Capreolus capreolus Austria
Liver	n.i.	0.005-0.50	n.i.	0.077-0.108	n.i.	Bilandžić et al. (2009) Cervus elaphus Croatia
Kidney		2.28-5.91		0.058-3.77		
Muscle		0.005-0.80		0.04-6.69		
Liver	n.i.	0.08-0.79	0.01-0.03	n.i.	n.i.	Petrović et al. (2013) Cervus elaphus Serbia
Kidney		0.03-4.99	0.01-0.10			
Muscle		n.i.	n.i.			
Liver	n.i.	n.i.	n.i.	<0.05-9.30	n.i.	Pokorny (2000) Capreolus capreolus Slovenia
Kidney	< 0.02-0.43		<0.01-0.69	n.i.		
Muscle	n.i.		n.i.	n.i.		

Legend: n.i.- not investigated; **(dry weight)*

Similar results for Cd and Pb distribution were obtained by other authors, but our results for Cd concentrations are markedly lower as compared with other investigations (Table 3). Since the age was not taken into account in data interpretation in the majority of cited studies (Table 3), the levels of toxic element could not be directly compared with our research. Secondly, the bioindicative approach was emphasized in our investigation, but many of cited studies were chosen in the vicinity of local pollution sources (smelters, industrial and mining areas). Finally, some differences between species must be outlined. Red deer (*Cervus elaphus)* whose anatomy of the digestive tract is adjusted to the intake of wide spectrum of feed, receives more wood species (35% of the feed in summer, 30% of the feed amount in winter). Roe deer whose digestive tract is only poorly adjusted to the digestion of cellulose, prefers young summer plants, herbs, fruits (they have narrow leaves and retain less falling particles) but more wide-leaved feed (herbs, summer plants which retain more deposited pollutants) than the red deer. This fact is likely to be the main reason of the inter-species differences (Kottferová and Koréneková B., 1998).

CONLUSIONS

Average as well as maximum concentrations of toxic elements in the majority tissue samples examined during our investigation did not exceed the maximum permissible levels and the levels measured in tissues of European free-living roe deer. The levels of investigated toxic elements are well below the concentrations considered dangerous for animal's health. However, high concentrations of some toxic elements still confirm the imperative of imposing relevant control program that will include veterinary officials as well as hunters and other subjects involved in game meat chain.

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