Heavy Metal Concentrations in Tissues of Wild Boar of Continental Croatia

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***Abstract*-Concentrations of heavy metals Cd, Hg and Pb were studied in the kidney and muscle tissues of free-living wild boars from three regions of continental Croatia. Significant iinterregional differences in kidney and muscle for Cd and Pb concentrations were determined. On the other hand, all three studied regions had similar concentrations of Hg in muscle and kidney. Obtained Cd concentrations were higher than those found in other countries. However, Pb levels shown lower values in comparison to other studies. Toxic elements levels, especially Cd in kidney of wild boar, suggest a detailed investigation of physiological and environmental factors contributing to animal contamination and an intensified control of meat and elimination of organ from human nutrition.**

Keywords-Metal Pollution; Cadmium; Lead; Mercury; Wild Boars; Croatia

I. INTRODUCTION

Over the last few decades everyday usage of heavy metals for industrial and agricultural purpose has increased their accumulation in soil, atmosphere and waters, foods and plants causes their incorporation into the food chain [1]. Wild free-living animals have an important role in the assessment of environmental pollution and are convenient bio-indicators of heavy metal pollution regarding to relatively long life span and integrating effects of environmental stress. Therefore it is provide an early warning of adverse toxic effects to the all compartments of ecosystems they belong. It is shown that accumulation of toxic heavy metals in plants and soil [2-7] may increase the risk of transfer to herbivorous wild mammals and game animals [8-11].

Heavy metal Cd, Hg and Pb are highly toxic may has adversely affects to the nervous, hematopoietic, endocrine, renal, nervous and reproductive systems, and may cause cardiovascular and pulmonary diseases, carcinogenicity, nephrotoxicity and neurotoxicity in man and animals [12, 13]. It is known that lead and cadmium may have important consequences for domestic and wild animals and for humans through acute or chronic exposure because accumulate in almost all tissues [14]. As reported in Spain Mediterranean forest ecosystem, these two metals are the most common toxic metallic pollutants in the liver and kidney of wild boar *(Sus scofra baeticus)* and red deer *(Cervus elaphus)* [15-19]. Study in Spain has suggested that Pb accumulates to a greater rate in wild boar and Cd in deer [18]. Also, recent investigations regarding to increased levels of Pb in tissues of deer and in red deer in Spain shown that contamination reflect mining and smelting activities [20, 21]. Increased presence of Pb minerals in the soils of this area originating from the mineralised underlying geology, and/or originating from the many abandoned mine workings in this area [22].

Cadmium substantially increase in environment through the industrial production of plastics, paints, dyes, dry batteries, and so on, by natural emissions of Cd, and also by usage of phosphate Cd containing fertilizers [14, 23]. The presence of Hg indicates environmental pollution from natural and anthropogenic sources. The main anthropogenic sources of Hg in the environment are smelting of non-ferrous metals, municipal waste incineration, coal combustion, paper industry and agriculture [24]. Consequently, Hg accumulates in different animal tissues and organs and causes contamination in food chain [25].

The aim of the present study is to investigate concentration of environmental contaminants Cd, Hg and Pb in muscle and kidney tissues of free-living wild boars in continental Croatia as important information about risk assessment in wildlife.

II. METHODOLOGY

A. Study Area

Muscle and kidney samples of wild boars *(Sus scrofa)* shot by hunters were collected in three regions of continental Croatia (Fig. 1). The animals were not selected according to sex or age but on the acknowledged assumption that they were aged from 2 to 5 years.

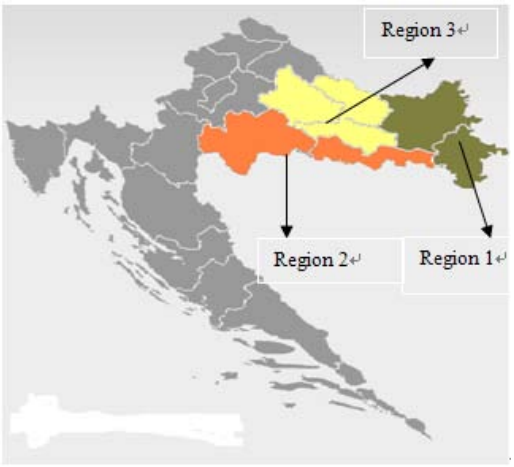

Fig. 1 Three studied regions of continental Croatia

Region 1 is consists of the most eastern counties Vukovar-Srijem and Osijek-Baranya. Two counties located

on the river Sava, Sisak-Moslavina and Brod-Posavina constituted Region 2. Region 3 is located between Regions 1 and 3 and is consist of three counties, namely Bjelovar-Bilogora, Požega-Slavonia and Virovitica-Podravina.

B. Sampling

In total 159 wild boar kidney and muscle samples were collected. Muscle samples were collected from the upper hind legs. Upon collection, all samples were placed into labelled plastic bags and stored at -18 °C to avoid tissue degradation prior to analysis.

Samples (2 g) were digested with 5 ml of HNO_3 (65% v/v Analytical Grade, Kemika, Croatia), 1 ml of H_2O_2 (30% v/v Analytical Grade, Kemika, Croatia) and 4 ml of with double deionised water (Milli-Q, 18.2 MΩ cm^{-1} resistivity) with a Multiwave 3000 microwave closed system (Anton Paar, Germany). A blank digest was carried out in the same way. The program for the digestion began at a potency of 1200 W then ramped for 10 min up to 180 °C, after which samples were held for 10 min at 1200 W at a temperature of 180 °C. Digested samples were diluted to a final volume of 50 ml with double deionised water.

C. Metal Analysis

Graphite furnace-atomic absorption spectroscopy using an AAnalyst 800 atomic absorption spectrometer (Perkin Elmer, USA) equipped with an AS 800 autosampler (Perkin Elmer, USA) were used for analyses of Cd and Pb. As matrix modifiers in each atomization 0.005 mg Pd and 0.003 mg Mg(NO3)2 were utilising. Mercury was analyzed by the cold vapour technique with a flow injection system coupled to an atomic absorption spectrophotometer FIAS-100 equipped with an autosampler AS 93 plus (Perkin Elmer, USA).

Calibrations were prepared from commercial solutions in HNO3 (0.2 %) with 1000 mg/l of each element (Perkin Elmer, USA). Detection limits were determined as the concentration corresponding to three times the standard deviation of twenty blanks. All specimens were run in batches that included blanks, a standard calibration curve, two spiked specimens, and one duplicate. The limits of detection (LODs, mg/kg) were: in muscle, for Cd 0.0004, for Pb 0.005 and 0.0004 for Hg; in kidney for Pb 0.0052, for Hg 0.0006 and 0.0005 for Cd. A reference sample of bovine liver (BCR 185R, Community Bureau of Reference) was analyzed (n = 5) and the recovery (mean % recovery ± S.E.) was 95.9 ± 6.2 % for Pb, 102.9 ± 5 % for Cd. There is no Hg certified value available.

III. STATISTICAL ANALYSIS

Statistical analysis was performed by the 6.0 Statistica® software package (StatSoft®, Inc., USA). Data were grouped according to tissue and sampling area. Concentrations were expressed as mean ± standard error (SE), minimum and maximum values. The *t*-test for independent samples was used to examine differences between tissues. To examine differences between sampling areas we used the one-way analysis of variance (ANOVA) test. Statistical significance was set at $p < 0.05$.

IV. RESULTS

In Tables Ⅰ, Ⅱ and Ⅲ were presented concentrations of Cd, Pb and Hg in wild boar muscle and kidney tissues in three regions of continental Croatia. The mean concentrations of Cd and Hg in the kidney of three regions were significantly higher than those found in muscle tissues ($p < 0.001$, respectively). For Pb significantly higher concentrations in kidney were measured than those in muscle tissues in Regions 1 ($p < 0.05$) and 2 ($p < 0.01$), while in Region 3 there was no differences. Regional differences between tissue concentrations of Cd and Pb were detected.

TABLE Ⅰ CADMIUM CONCENTRATIONS IN THE MUSCLE AND KIDNEY OF WILD BOARS IN THREE CONTINENTAL REGIONS OF CROATIA

Region	Matrix	N	Mean± SE (mg/kg)	Range (mg/kg)
1	Muscle	53	0.026 [a] ± 0.008	0.001 – 0.428
	Kidney		2.65 ± 0.41	0.003 – 11.2
2	Muscle	51	0.005 [ab] ± 0.001	0.001 – 0.027
	Kidney		2.95 ± 0.36	0.36 – 13.7
3	Muscle	55	0.037 [b] ± 0.007	0.001 – 0.32
	Kidney		2.91 ± 0.38	0.09 – 11.5

Different letters indicate significant differences among muscle tissues ([a]$p<0.01$; [b]$p<0.001$)

Significantly higher Cd levels were found in Region 1 ($p < 0.01$) and Region 3 ($p < 0.001$) in comparison with Region 2. However, there were no significant differences in kidney Cd levels between three regions.

TABLE Ⅱ LEAD CONCENTRATIONS IN THE MUSCLE AND KIDNEY OF WILD BOARS IN THREE CONTINENTAL REGIONS OF CROATIA

Region	Matrix	N	Mean± SE (mg/kg)	Range (mg/kg)
1	Muscle	53	0.032 [aa] ± 0.007	0.001 – 0.31
	Kidney		0.10 [c] ± 0.032	0.001 – 1.63
2	Muscle	51	0.067 [a] ± 0.016	0.001 – 0.57
	Kidney		0.28 [c] ± 0.083	0.015 – 3.89
3	Muscle	55	0.096 [a] ± 0.029	0.002 – 1.01
	Kidney		0.17 ± 0.042	0.01 – 1.83

Different letters indicate significant differences among muscle tissues ([a]$p<0.05$) and kidney tissues ([c]$p<0.01$)

TABLE Ⅲ MERCURY CONCENTRATIONS IN THE MUSCLE AND KIDNEY OF WILD BOARS IN THREE CONTINENTAL REGIONS OF CROATIA

Region	Matrix	N	Mean± SE (mg/kg)	Range (mg/kg)
1	Muscle	53	0.009 ± 0.001	0.0002 – 0.036
	Kidney		0.076 ± 0.008	0.001 – 0.277
2	Muscle	51	0.008 ± 0.001	0.001 – 0.061
	Kidney		0.093 ± 0.019	0.01 – 0.983
3	Muscle	55	0.007 ± 0.001	0.001 – 0.04
	Kidney		0.063 ± 0.011	0.09 – 0.545

Regional differences between muscle tissues shown significantly lower Pb concentrations in Region 1 than those in Region 2 and 3 ($p < 0.05$, both). Also, significantly higher Pb levels were found in Region 2 than in Region 1 ($p < 0.05$).

V. DISCUSSION

The Cd, Pb and Hg concentrations reported in previous studies in Croatia and other European countries in wild boars were presented in Table Ⅳ. Mean concentrations of Cd measured in the kidney of present study were consistent in all three regions observed and being significantly higher than those found in the muscle in the same species. In comparison with Cd levels from other countries mean kidney Cd concentrations were more than 2-fold higher than those measured in animals from Spain [18] or more than 10-fold higher than in Slovakia [8]. However, present results were 1.2- to 4-fold smaller in comparison with previous findings in earlier years in Croatia [26]. Interregional differences were observed for muscle Cd and the lowest levels were determined in Region 2.

TABLE Ⅳ CONCENTRATIONS OF CD, PB AND HG IN TISSUES OF WILD BOARS IN OTHER COUNTRIES

Country / Year	Tissues	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)
Slovenia / 1995[a]	kidney	2.429	1.62	
Slovakia / 1998[b]	muscle	0.02	0.28	
	kidney	0.24	0.25	
Spain / 1998[c]	kidney	1.35	0.62	
Poland / 2002[d]	kidney			0.04 – 0.015
Croatia / 2009[e]	muscle	0.01-0.23	0.059 - 2.28	
	kidney	1.04 – 5.98	0.073 - 0.355	

[a] [28]; [b] [8]; [c] [18]; [d] [29]; [e] [26]

According to the Croatian regulations [27] for Cd, Pb and Hg in the meat and kidney of animals intended for human consumption, the maximum allowed concentration of Cd is 0.05 mg/kg in muscle and 1 mg/kg in kidney; the respective level of Pb is 0.1 mg/kg in muscle and 0.5 mg/kg in kidney. There were no specified concentrations for Hg in tissues of animals.

According to mean values obtained for muscle and kidney tissues Cd concentrations in muscle were below allowed concentration. However, mean kidney Cd levels exceeded the maximum allowed concentration in all three regions.

In present study muscle Pb concentrations were 3- to 5.7-tenfold [8] and 16-fold [15] lower than those measured in animals from Slovakia and Poland. However, renal Pb levels were 1.6- to 10-fold lower than levels found in Spain [18] and also from 5.7- to 16-fold lower than those in animals from Slovenia [28], but similar to previous findings in earlier years in Croatia [26].

Regional differences in Pb concentrations for muscle and kidney tissues were observed in this study. Mean values of Pb obtained in muscle and kidney tissue were below allowed concentration 0.1 mg/kg and 0.5 mg/kg according to Croatian regulations.

In previous studies regional differences in Cd and Pb concentrations in kidney of wild boars were also found [18, 28, 30, 31]. Three regions studied had no mining activities, industrial production or pollution sources to yield the accumulation of Cd, Pb and Hg and contribute to contamination of tissues in free-living animals which is recorded in European countries such as Slovenia [28, 32], Spain [18, 21], Germany [33], Netherlands [34] and Poland [10, 16, 29, 35].

All three studied regions had similar concentrations of Hg in muscle and kidney tissues. The mean Hg levels found in this study were for more than 10-fold higher than previous findings in the kidney of wild boars in Poland [29]. Mean concentrations measured not exceeding allowed levels of 0.03 mg/kg in muscle and 0.1 mg/kg in kidney.

Lead and cadmium are present in all ecosystems and wild boar is an omnivorous species and free-migrating animal which can move long distances through a day so integrating the contamination of large areas. The concentration of a contaminating metal in specific animal tissue depends on the rate and duration of intake by the individual. Important exogenous factors of elevated levels of these metals in kidney of wild animals are feed, geographical origin, relief, wind drifts, growth patterns and seasonal variations [8, 14, 21, 35]. In further studies to find the source of contamination of wild boar different factors such as age, seasonal variations, food preferences and diet composition have to be studied.

VI. CONCLUSION

The concentrations of Cd and Hg in the kidney of wild boar were higher than those found in the muscle tissues. The results also show Cd and Pb concentrations differences in muscle and kidney between three regions studied.

Obtained Cd concentrations were higher than those found in other countries, while Pb levels show lower values in comparison to other studies. Furthermore, these findings point out the need to discover the causes of animal contamination and an intensified control of meat and elimination of kidney tissues from human nutrition.

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