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Cadmium concentrations in the tissues of young wild boars (*Sus scrofa* L.) from Moslavina and Slavonia in lowland Croatia

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ABSTRACT

Samples of skeletal muscles, liver and kidneys of young wild boars from Moslavina and Slavonia were analysed for cadmium content. Organ samples were taken from wild boars (up to 1 year old) in the 2007/2008 hunting season from the hunting grounds: Črnovščak (n = 8) and Spačva (n = 9). The highest value of cadmium measured in muscles was 0.0029 µg/g (Spačva), in the liver 0.0874 µg/g (Črnovščak) and kidneys 0.8509 µg/g (Črnovščak), which is the highest concentration of Cd from all studied tissues. All measured concentrations were lower than the reference values according to Croatian regulations for Cd in food. Cd concentrations in muscle tissue were much lower than the concentrations in the liver and kidney. Cadmium median values in the muscle tissue of wild boars from Moslavina (0.00065 µg/g) were lower than the values in wild boars from Slavonia (0.0008 µg/g), but not significantly, while the median concentrations of Cd in liver and kidney tissue were higher in wild boars from Moslavina (0.0483 µg/g and 0.5259 µg/g), in comparison with those in Spačva (0.0283 and 0.4770 µg/g). Additionally, statistically significant differences were only defined for liver tissue. The results of this research indicate that: 1. even young wild boars (aged up to 1 year old) can be bioindicators of environmental pollution with Cd; 2. The researched area Črnovščak is under stronger anthropogenic influence than the Spačva hunting ground; 3. the amount of Cd in the tissues is lower than the permitted concentration.

Key words: wild boars, cadmium, tissue concentrations, legislation

Introduction

The actual risk presented by toxic metals to wildlife and humans is mostly demonstrated as a chronic or sub-lethal effect, e.g. nephrotoxicity, carcinogenicity, teratogenicity, endocrine and reproductive toxicities (SREBOČAN and SREBOČAN, 2009;

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RANI et al., 2014). One of the most prominent heavy metals with this effect is cadmium, which is toxic in very small amounts, because of its limited biotransformation processes, which results in a slow excretion rate and accumulation in the living organism. Therefore, it is necessary and important to monitor the cadmium concentrations in the environment, in cities and industrial areas, as well as in areas that are not directly related to pollution sources. Although cadmium was for many years the subject of scientific research, it is still topical in ecotoxicological studies worldwide and in Croatia (BILANDŽIĆ et al., 2009; BILANDŽIĆ et al., 2010; SREBOČAN et al., 2011; DANIELI et al., 2012; GASPARIK et al., 2012; ŠURAN et al., 2013; SZKODA et al., 2013; YARSAN et al., 2104; DLUGASZEK and KOPCZYNSKI, 2014; LAZARUS et al., 2014). It is also important, based on the results of various forms of scientific research, to raise the level of environmental awareness in the total population, and bring about the enactment of legislation aimed at preserving natural resources for future generations. Wild animals, especially game species, such as wild boar, are suitable as bioindicators (FROSLIE et al., 2001) due to their large geographical distribution, residential way of life, feeding habits, relatively long life-span and easy sample collection (regular hunting activities). In the Republic of Croatia, wild boar are resident in all hunting areas and their meat is prized food in the human diet, hence it has to be safe and meet the quality requirements prescribed by legislation (EC, 2006). This is supported by the fact that the most frequently hunted large wild animals in 2011 and 2012 in Croatia were wild boars (CSB, 2013).

The aim of the present study was to analyse the cadmium concentrations in various wild boar tissues in relation to different living areas, and to determine whether young animals can serve as bioindicators of environmental pollution with Cd. Based on the parameters obtained, an evaluation of the environmental contamination of two Croatian territories are discussed, considering the possible different anthropogenic impact in these areas, as well as its potential negative effects on wild boar and subsequently human health status. Our results are compared with related previous and recent published data from Croatia and some European countries.

Materials and methods

Samples were collected during a regular hunting season (2007-2008) from two different open state hunting grounds in lowland Croatia, first: in Moslavina: the Črnovščak hunting ground No. I/3 covering an area of 2158 ha, which is situated in the wider area of the village Prečec, near the green route in the eastern part of Zagreb County. It is located about 20 km east of Zagreb, along the River Lonja between Dugo Selo and Ivanic Grad; and second: in Slavonia, the Spačva hunting ground No. XVI/11 - 25018 ha, which is located south of Vinkovci and east of Županja, in the area of the Slavonian plains, at an latitude of 77-90 meters, between the Danube and Sava rivers, crisscrossed by numerous water areas: the rivers Spačva, Bosut and Virovi (Fig. 1).

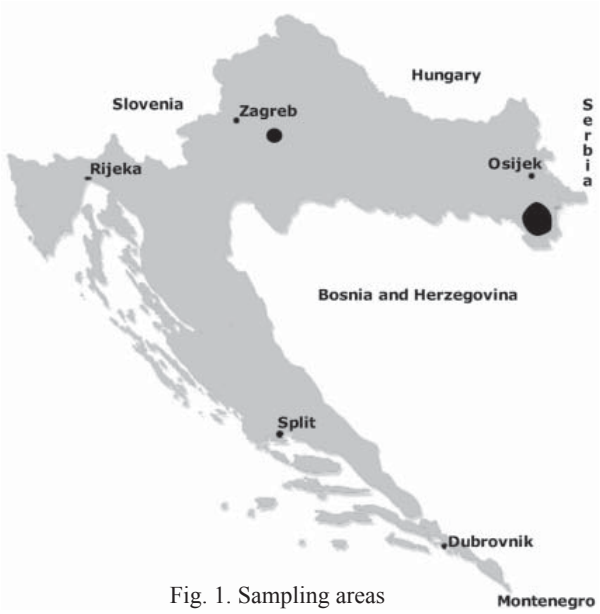

Fig. 1. Sampling areas

Table 1. Data on wild boars in the study

No	sex	Age months	Body mass kg	Location of catch
1	♂	5-6	30	Črnovščak
2	♂	5-6	30	Črnovščak
3	♂	5-6	35	Črnovščak
4	♂		20	Črnovščak
5			30	Črnovščak
6			30	Črnovščak
7			20	Črnovščak
8			20	Črnovščak
9	♀	6		Spačva
10	♂	12		Spačva
11	♂	12		Spačva
12	♂	12		Spačva
13	♂	12		Spačva
14	♀	12		Spačva
15	♀	12		Spačva
16	♀	12		Spačva
17	♀	12		Spačva

All shot animals were aged on the basis of the guidelines given by the specific literature (WAGENKNECHT, 1984). In relation to the place of shooting, the animals were divided into two groups: young wild boars from the Moslavina (n = 8) and Slavonia areas (n = 9). Information about the animals from which tissue samples were taken is shown in Table 1.

Samples of muscle, liver and kidney tissue were packed separately in polyethylene bags, frozen, properly signed and stored at -20 °C until analysis. Before analysis, the samples were thawed slightly and rinsed with deionized water. Approximately 1.5 g of the muscle, liver and kidney homogenate were weighed for cadmium determination, dried at 105 °C and dry ashed overnight in quartz crucibles in a muffle furnace at 450 °C. The ash residues were then dissolved in concentrated nitric acid, heated and filled up to 10 mL with deionized water. Cadmium was measured by Perkin Elmer Analyst 600 atomic absorption spectrometry (Shelton, USA), equipped with a transversely heated graphite furnace unit, autosampler and longitudinal Zeeman effects background correction. Argon was used as the purge gas. A mixture of magnesium nitrate and palladium nitrate was used as matrix modifier. At least two replicate determinations were made for each sample. The certified reference material was used in duplicate, together with each sample series for method validation, in order to check the accuracy: bovine liver 1577b (National Institute of Standards and Technology, USA) for Cd. The result was as follows (mean ± SD; µg/g wet wt.; n = 7): 0.484 ± 0.040 for Cd (certified value: 0.500 ± 0.03 µg/g wet wt).

The results were processed by Statistica 8.0 software (StatSoft Inc. 2009). For all measured concentrations of Cd in muscle, liver and kidney tissue, median values and the values of the upper and lower quartiles were calculated. The significance of differences between the mean values of the same tissue samples from the boar from the two different areas was calculated by Student's *t*-test, with differences at the level of $P<0.05$ considered significant.

Results

The individually measured Cd concentrations (µg/g) in muscle, liver and kidney tissue are shown in Table 2. Median values, lower and upper quartiles and min. and max. values of Cd in the muscle, liver and kidney tissues of wild boars from Moslavina and Slavonia are presented in Figs. 2, 3 and 4, respectively.

The max Cd concentration in the muscle samples from Moslavina, measured in sample No. 8, was 0.0027 µg/g; in liver sample No. 2 (0.0874 µg/g), and in the kidney, where the largest accumulation was found in sample No. 1 (0.8509 µg/g). This is the highest measured tissue concentration of Cd.

In samples from the area of the Spačva hunting ground (Slavonia), the highest values were recorded in muscle sample No. 13 - 0.0029 µg/g; liver sample No. 10 - 0.066 3 µg/g, and in the kidney in the sample No. 15. at amounts to 0.7446 µg/g.

Table 2. The individually measured Cd concentrations (µg/g) in the muscle, liver and kidney tissues of wild boars from the Moslavina and Slavonia regions

No. sample	Cd µg/g		
	Muscle	Liver	Kidney
1	0.0017	0.0458	0.8509
2	0.0003	0.0874	0.6260
3	0.0005	0.0642	0.6954
4	0.0003	0.0710	0.6066
5	0.0008	0.0291	0.1790
6	0.0011	0.0223	0.1902
7	0.0003	0.0508	0.3844
8	0.0027	0.0396	0.4452
9	0.0006	0.0242	0.2638
10	0.0009	0.0663	0.6790
11	0.0005	0.0242	0.2404
12	0.0002	0.0158	0.1745
13	0.0029	0.0320	0.3475
14	0.0009	0.0317	0.4770
15	0.0010	0.0302	0.7446
16	0.0008	0.0238	0.5505
17	0.0008	0.0283	0.5621

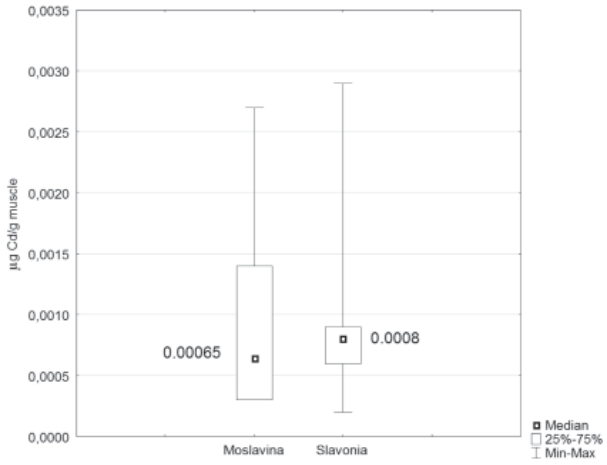

Fig. 2. Median cadmium concentration in muscle tissue (µg/g) of young wild boars from Moslavina and Slavonia

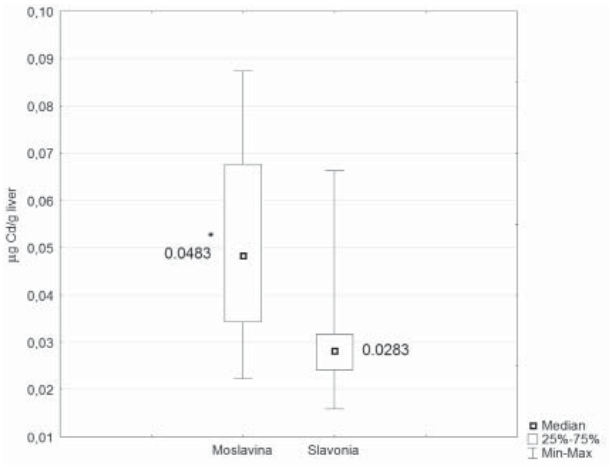

Fig. 3. Median cadmium concentration in liver tissue (µg/g) of young wild boars from Moslavina and Slavonia

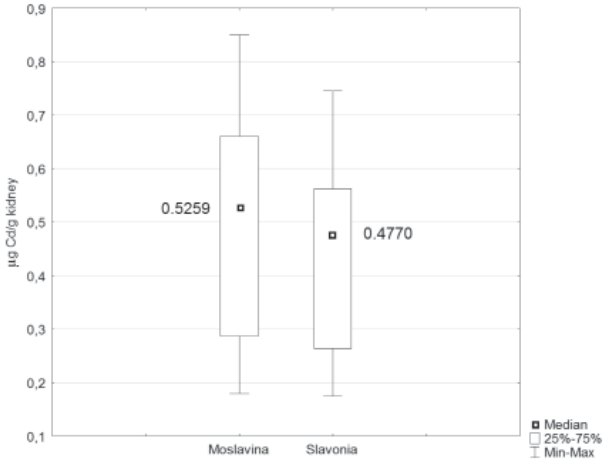

Fig. 4. Median cadmium concentration in kidney tissue (µg/g) of young wild boars from Moslavina and Slavonia

Discussion

Although the wild boar organ samples were taken from animals under the age of one year, we confirmed the presence of cadmium in all investigated tissues. This means that they had already come into contact with contaminated food and water, or cadmium in the air. It is possible that piglets, which suckle for about 3 months, were exposed to cadmium through colostrum or milk. For a more accurate conclusion about the origin of cadmium in the tissues of young wild boars we need much more data about their mothers, as well as about the uptake of cadmium from milk to piglets. Wild boars are omnivores that feed on different plant foods: grains, herbs, mushrooms, forest fruits (up 80-90 % of the diet) and food of animal origin: caterpillars, larvae, carcass and small animals (13 %). In the environment, cadmium is predominantly bound to the soil; it is highly mobile and readily enters the food chain through land plants and animals (CHANEY et al., 1987). Cadmium concentrations measured in muscles were relatively low (max. 0.0029 μg/g), higher in the liver (max. 0.0874 μg/g) and highest in the kidneys (max. 0.8509 μg/g), which is in accordance with the well-known Cd distribution pattern. Muscle cadmium concentrations were several times lower than those in liver and kidney tissue, which is in agreement with all the cited research into wild boars from Europe and Croatia. From Table 3, which shows the individual values of cadmium in piglets, it can be seen that the maximum value for muscles was measured in sample No. 13, for livers in sample No. 2 and for kidneys in sample No. 1. Since the highest measured concentrations of cadmium in different tissues were not determined in the same individual, this finding indicates unsystematic contamination with the metal. The presence of cadmium in 83 % of samples of kidney tissue at a concentration more than 10 times higher than that in the soil (HALAMIĆ and MIKO, 2009) supports the tendency of cadmium accumulation in the body already in the first year of life. Although young and short-lived animals are recommended for monitoring programs of environmental pollution by substances that are not persistent in the environment, or to indicate contamination levels occurring just before collection (MOORE, 1966), our results indicate that young boars may be indicators of contamination by cadmium.

The results of this study are based on samples obtained from individuals from the Moslavina area and from the region of Slavonia. Both regions are characterized by intensive agricultural production, and therefore the use of the various pesticides and fertilizers, but there is no industrial activity there. Nevertheless, according to the Geochemical Atlas of the Republic of Croatia, cadmium concentrations in soil from both the investigated areas are equal and low (median 0.2 μg/g d/w) (HALAMIĆ and MIKO, 2009). Median concentrations of cadmium in the muscle tissue of boar from the Moslavina area were somewhat lower than in the young from Slavonia, but not significantly (Fig. 2). In contrast, median cadmium concentrations in liver and kidney tissues (Fig. 3, and 4)

were higher in boar from the Moslavina area, compared to those from Spačva. Statistically significant differences were found for liver tissues. These results could be associated with pronounced human impact, such as proximity to industrial areas and intensive agricultural production, because the Črnovščak hunting ground is located about 20 km east of Zagreb, and in the immediate vicinity there is a railway line and the A3 motorway. It is known that cadmium in fossil fuels, motor oil, and tyres also represents a source of pollution of the soil, vegetation and fauna along highways and near airports (GISH and CHRISTENSEN, 1973). Of industrial facilities, it is important to note the proximity of the INA oil industry plant and supporting chemical production industries (Ivasim) and gas production (Ethan). Within the hunting area there is a cattle farm at Božjakovina. On the other hand, the Spacva hunting ground is located on the Slavonian plains between the Danube and Sava rivers, crisscrossed by numerous water surfaces. It is an open hunting ground within the largest continuous complex of Slavonian oak forests in Europe, and it is an ideal habitat for deer, roe deer, wild boar, waterfowl and meadow grass. Also, the main road route that connects Europe and the Middle East - the A3 motorway, a railway line and a number of local roads pass through this area. Median cadmium concentrations in the muscle and liver tissue of young wild boars from both investigated areas are comparable with previous published data from lowland Croatia (SREBOČAN et al., 2011; ŠURAN et al., 2013), if we take into consideration the age of the animals, but in the kidneys they were lower than in other parts of continental Croatia (BILANDŽIĆ et al., 2009; BILANDŽIĆ et al., 2010; ŠURAN et al., 2013). Also, the tissues of wild boars from our study were less contaminated with cadmium than boars from Poland (FALANDYSZ et al., 1986; FALANDYSZ et al., 1987; FALANDYSZ and LORENC-BIALA, 1988; FALANDYSZ, 1994), from Germany (HECHT, 1986; RIMKUS and WOLF, 1987; LUSKY et al., 1992; WILKE, 2000), from Spain (SANTIAGO et al., 1998), the Netherlands (WOLKERS et al., 1994; KUITERS, 1996), Austria (TATARUCH, 1989; TATARUCH, 1993), Slovakia (PISKOROVA et al., 2003), and from Karelian wild boars (MEDVEDEV, 1999). In only two papers were lower kidney cadmium concentrations recorded than those from our study: from Hungary (SOMLYAY et al., 1983) and Slovakia (KOTTFEROVA and KORENEKOVA, 1989). Precise comparison of the results is not always possible, as many authors do not divide the animals according to age, which is of particular importance for interpretation of the results, because of the known second regularity of cadmium - the positive correlation between cadmium concentrations and age.

In the Croatian regulations on maximum permitted levels (ML) of metals, which have been harmonized with EU legislation (EC, 2006), cadmium ML is only regulated for farmed animals (i.e. cow, sheep, pig, and poultry). Comparing our results with the current ML values, where fresh meat may contain up to 0.05 μg/g, liver 0.5 μg/g, and kidneys 1.0 μg/g of cadmium, we may conclude that our values are lower than those prescribed. Considering the fact that the amount of cadmium in the meat, but also in the offal of young

wild boar, is not greater than the ML, these foods may be considered suitable for human consumption. Although there is a general recommendation for consumers to avoid eating the offal of wild animals in Croatia, because of their high Cd content (CFA, 2012; NRMP, 2012), our results suggest that this is not necessarily the case for the liver and kidneys of young wild boars. In the report by the Central Bureau of Statistics of the Republic of Croatia (CBS, 2013) for the period 1999-2011, it was specified that 0.43 kg of game and rabbit meat/year/ person (8 g week/person) and 1.10 kg game and farmed animal offal/ year/person (21 g week/person) were consumed in Croatian households. This means that game meat consumption in Croatia is less than 1 % of the total annual consumption of meat per person. In other European countries this percentage is somewhat higher, but it should be emphasized that these are ingredients that consumers consider to be a delicacy, and despite the small quantities, they are important as part of the tourist attractions of the country. In recent years, hunting tourism has been evolving in Croatia by promoting the natural beauty, which leads to the obligation for the safety control of game meat as a food of animal origin.

On the basis of these results it may be concluded that wild pigs at the age of one year may already be a bioindicator of environmental pollution by cadmium, although for an overall estimate of specific area pollution, the known fact that cadmium tends to accumulate in the body should be considered, i.e. Cd concentration in tissues is always higher in older animals.

The finding of slightly higher concentrations of cadmium in the tissues of young wild boars from Moslavina suggests a slightly higher anthropogenic influence in relation to Slavonia, but it is mostly not significant. Cadmium in all tissues was far below those concentrations that may cause harmful effects on the health of wild pigs. Also, from the hygienic point of view, the muscle, liver and kidney tissues of young wild boars are safe for human consumption, since the concentrations of cadmium did not exceed values prescribed by the official regulations. The results of this research are a contribution to ecotoxicological research in the area of lowland Croatia, and a basis for comparing the results of future research from two other characteristic game habitats present in Croatia, namely karst and Mediterranean habitats.

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PREVENDAR CRNIĆ, A., J. ŠURAN, H. CIPRIŠ MADUNIĆ, F. BOŽIĆ: Koncentracija kadmija u tkivima mladih divljih svinja (*Sus scrofa* L.) s područja Moslavine i Slavonije u nizinskoj Hrvatskoj. Vet. arhiv 85, 323-334, 2015.

SAŽETAK

Koncentracija kadmija određena je u uzorcima mišićnog, jetrenog i bubrežnog tkiva mladih divljih svinja s područja Moslavine i Slavonije. Uzeti su uzorci organa divljih svinja (u dobi do godine dana), u lovnoj sezoni 2007./2008., u lovištima Črnovščak (n = 8) i Spačva (n = 9). Najviše izmjerene vrijednosti kadmija bile su: u mišiću 0,0029 µg/g (Spačva), u jetri 0,0874 µg/g (Črnovščak) i bubregu 0,8509 µg/g (Črnovščak), što je i najviša izmjerena koncentracija Cd u ovom istraživanju. Sve dobivene vrijednosti bile su niže od onih koje propisuje Pravilnik o najvišim dopuštenim koncentracijama kadmija u hrani. Kadmij u mišićju bio je u mnogo nižoj koncentraciji u odnosu na bubrežno tkivo. Medijana vrijednost kadmija u mišićju divljih svinja iz Moslavine (0,00065 µg/g) bila je niža od vrijednosti u divljih svinja iz Slavonije (0,0008 µg/g), ali ne statistički značajno, dok su medijane koncentracije Cd u jetrenom i bubrežnom tkivu bile više u divljih svinja iz Moslavine (0,0483 µg/g i 0,5259 µg/g), u odnosu na one iz Spačve (0,0283 i 0,4770 µg/g). Pritom je statistički značajna razlika utvrđena samo za jetreno tkivo. Rezultati ovog istraživanja upućuju na: 1. da mlade divlje svinje (u dobi do 1 godine) mogu biti bioindikatori kontaminacije okoliša kadmijem; 2. da je istraživano područje Črnovščak pod izrazitijim antropogenim utjecajem u odnosu na lovište Spačva; 3. da su koncentacije kadmija u tkivima mladih divljih svinja ispod onih propisanih pravilnikom.

Ključne riječi: divlja svinja, kadmij, koncentracija u tkivu, zakonska regulativa