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Wild Boar Tissue Levels of Cadmium, Lead and Mercury in Seven Regions of Continental Croatia

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Abstract Concentrations of cadmium, mercury and lead were analysed by atomic absorption spectrometry in the kidney and muscle of free-living wild boar (n = 169) from hunting grounds in seven counties of continental Croatia. Mean levels of metals (mg/kg) in muscle and kidney of boars ranged as follows: Cd: 0.005–0.016 and 0.866–4.58, Pb: 0.033–0.15 and 0.036–0.441, Hg: 0.004–0.012 and 0.04–0.152. In all seven regions, concentrations exceeded the permitted values (muscle and kidney mg/kg: cadmium 0.05/1; lead 0.1/0.5; mercury 0.03/0.1) in 13.6% and 71.6% of samples (muscle and kidney, respectively) for cadmium; 13.6% and 8.9% for lead; 19.5% and 2.4% for mercury. There were significant differences among the regions. Vukovar-Srijem and Virovitica-Podravina Counties were highly contaminated with cadmium, Sisak-Moslavina and Virovitica-Podravina Counties with lead and Brod-Posavina County had highest mercury concentrations. These results suggest a detailed investigation of physiological and environmental factors contributing to accumulation of metals in boars.

Keywords Cadmium · Lead · Mercury · Wild boars

Constant human impacts on the environment through the daily use of heavy metals in industrial production and for agricultural purpose have increased in recent decades, and their accumulation in the environment has increased accordingly. Wild mammals are convenient indicators of heavy metal pollution due to their relatively long lifespan, and thus long period of accumulating chemicals and thus they provide an early warning of adverse toxic effects in the ecosystem as a whole. Heavy metals and metalloids such as cadmium (Cd), mercury (Hg) and lead (Pb) are highly toxic and may induce immunosuppression, impaired reproduction and teratogenicity, damage to the nervous system, cardiovascular and pulmonary diseases, carcinogenicity, nephrotoxicity and neurotoxicity (Satarug et al. 2003). The accumulation of toxic heavy metals in plants and soil (Tyler 2001) may increase the risk of transfer to herbivorous wild mammals and game animals or to livestock (Falandysz et al. 2005; Wlostowski et al. 2006).

Lead is a natural constituent of the biogeosphere but also enters the environment from incinerators of solid waste, metal smelters, coal-fired power stations, sewage sludge or waste oil. However, the dominant anthropogenic emission of Pb into the environment results from the use of organolead additives to gasoline (Nriagu 1989). Recent investigations have shown that Pb contamination in deer (Beyer et al. 2007) and in red deer (Reglero et al. 2008) may be due to mining and smelting activities. Mercury accumulates in different animal tissues and organs and causes contamination in the food chain. The presence of mercury is an indication of environmental pollution from natural and anthropogenic sources such as smelting of non-ferrous metals, municipal waste incineration, coal combustion, paper industry and agriculture (Dietz et al. 1996). Through the industrial production of plastics, dry batteries, paints, dyes, etc., natural emissions of Cd, and the use of phosphate fertilizers containing significant quantities of Cd, environmental concentrations of this metal are

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significantly increasing (Satarug et al. 2003). Following oral exposure to Cd in food and drinking water, it is preferentially accumulated in the liver and kidneys of wild animals (Santiago et al. 1998; Wlostowski et al. 2006).

The primary objective of this study was to provide new data on the concentration of environmental contaminants Cd, Hg and Pb in kidney and muscle tissues of free-living wild boars in seven regions of continental Croatia, as important information in conducting risk assessments for wildlife and for humans.

Materials and Methods

Samples of muscle and kidney of wild boar (*Sus scrofa*) shot by hunters were collected from seven counties of continental areas of Croatia located between the Sava and Drava Rivers (Fig. 1): Sisak-Moslavina (SM), Brod-Posavina (BP), Vukovar-Srijem (VS), Bjelovar-Bilogora (BB), Požega-Slavonia (PS), Virovitica-Podravina (VP) and Osijek-Baranja (OB).

Sampling of the wild animal organs was performed pursuant to the National Residue Monitoring Plan of the Republic of Croatia during the 2008/2009 hunting season. Earlier reports of elevated Cd and Pb levels in the kidney and muscle of wild boar resulted in an intensive survey of these metals in the respective hunting areas in seven Croatian counties. Animals were not selected according to sex or age but on the acknowledged assumption that they were aged from 2 to 5 years. Thus, kidney and muscle samples were collected from each animal (total of 169 animals). 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.

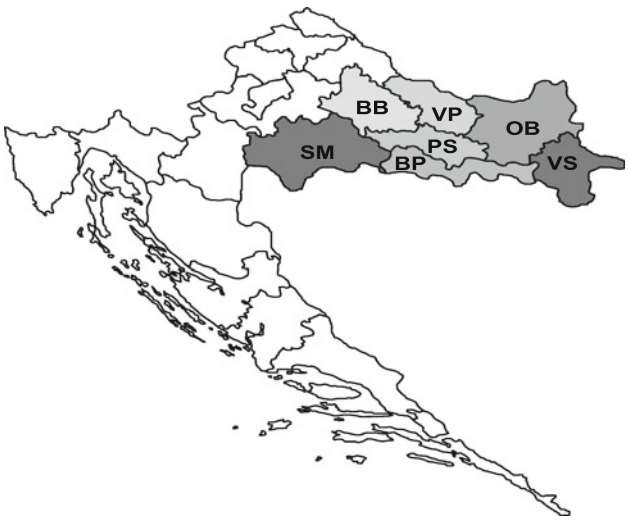

Fig. 1 Seven study areas in continental Croatia (*SM* Sisak-Moslavina, *BP* Brod-Posavina, *VS* Vukovar-Srijem, *BB* Bjelovar-Bilogora, *PS* Požega-Slavonia, *VP* Virovitica-Podravina *OB* Osijek-Baranja)

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 H_2O (Milli-Q grade) with a microwave oven (Anton Paar Multiwave 3000). The digestion program began at a potency of 1,200 W then was ramped for 10 min to 180°C, after which samples were held at 1,200 W and a temperature of 180°C for 10 min. Digested samples were diluted to a final volume of 50 mL with Milli-Q H_2O.

Analyses of Cd and Pb were conducted by graphite furnace-atomic absorption spectroscopy using an Analyst 800 (Perkin Elmer) equipped with an autosampler AS 800 (Perkin Elmer) and utilizing 0.005 mg $Pd(NO_3)_2$ and 0.003 mg $Mg(NO_3)_2$ as matrix modifiers in each atomization for Pb and Cd. Mercury was analyzed using the cold vapor technique with a flow injection system coupled to an atomic absorption spectrophotometer FIAS-100 (Perkin Elmer) equipped with an autosampler AS 93 plus (Perkin Elmer).

Calibrations were prepared from commercial solutions in HNO_3 (0.2%) with 1,000 mg/L of each element (Perkin Elmer). Detection limits (LODs, in mg/kg dry weight, back-calculated to in tissue concentrations) were 0.0004 Cd, 0.005 Pb and 0.0004 Hg in muscle; 0.0052 Pb, 0.0006 Hg and 0.0005 Cd in kidney. Blanks were processed in each batch of digestions. A reference sample of bovine liver (BCR 185R, Community Bureau of Reference) was analyzed ($n = 5$) and the recovery (mean % recovery ± SE) was 95.9 ± 6.2% for Pb and 102.9 ± 5% for Cd (there is no Hg certified value available).

Statistical analysis was performed by the Statistica® software package (1999, StatSoft® Inc., Tulsa, USA). Data were grouped according to tissue and sampling area. Concentrations were expressed as mean ± standard error, 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 $p < 0.05$.

Results and Discussion

The concentrations of Cd, Pb and Hg in wild boar muscle and kidney tissues from seven different areas of continental Croatia are presented in Tables 1, 2 and 3. Mean Cd concentrations were significantly higher in kidney than in muscle tissues ($p < 0.0001$, respectively) in all seven counties. These results were consistent with previous reports in the same species (Doganoc and Šinigoj-Gačnik 1995). The average renal Cd levels in the present study

Table 1 Cadmium concentrations (mg/kg) in the muscle and kidney of wild boars from seven Croatian counties

County	Matrix	Number of animals	Mean ± S.E	Min–max	Permitted values	Samples exceeding permitted values (%)
SM	Muscle	31	0.005 ± 0.0107	0.001–0.027	0.05	0
	Kidney		2.49 ± 0.426	0.49–13.67	1	83.9
BP	Muscle	20	0.005 ± 0.0009	0.001–0.16	0.05	0
	Kidney		3.66 ± 0.616	0.357–10.56	1	90
VS	Muscle	26	0.041 ± 0.0164	0.0002–0.428	0.05	23.1
	Kidney		3.28 ± 0.621	0.167–11.21	1	80.8
BB	Muscle	23	0.021 ± 0.0071	0.002–0.151	0.05	17.4
	Kidney		0.866 ± 0.1125	0.098–2.29	1	30.4
PS	Muscle	17	0.015 ± 0.0056	0.001–0.068	0.05	12.5
	Kidney		3.33 ± 0.712	0.735–9.97	1	75
VP	Muscle	25	0.062 ± 0.0153	0.001–0.32	0.05	36
	Kidney		4.58 ± 0.612	0.11–11.49	1	88
OB	Muscle	27	0.011 ± 0.0042	0.001–0.113	0.05	4
All	Kidney		2.03 ± 0.507	0.003–9.31	1	55.6
	Muscle	169	0.023 ± 0.0039	0.0002–0.428	0.05	13.6
	Kidney		2.84 ± 0.216	0.003–13.67	1	71.6

SM Sisak-Moslavina, *BP* Brod-Posavina, *VS* Vukovar-Srijem, *BB* Bjelovar-Bilogora, *PS* Požega-Slavonia, *VP* Virovitica-Podravina, *OB* Osijek-Baranja

Table 2 Lead concentrations (mg/kg) in the muscle and kidney of wild boars from seven districts of Croatia

County	Matrix	Number of animals	Mean ± SE	Min–max	Permitted values	Samples exceeding permitted values (%)
SM	Muscle	31	0.092 ± 0.0233	0.003–0.57	0.1	32.3
	Kidney		0.441 ± 0.1301	0.025–3.89	0.5	22.6
BP	Muscle	20	0.028 ± 0.0139	0.001–0.279	0.1	5
	Kidney		0.036 ± 0.0614	0.015–0.128	0.5	0
VS	Muscle	26	0.04 ± 0.0123	0.002–0.31	0.1	11.5
	Kidney		0.101 ± 0.0279	0.02–0.706	0.5	3.8
BB	Muscle	23	0.067 ± 0.0307	0.002–0.71	0.1	13
	Kidney		0.173 ± 0.0301	0.028–0.65	0.5	4.3
PS	Muscle	17	0.033 ± 0.0062	0.002–0.096	0.1	0
	Kidney		0.195 ± 0.0868	0.023–1.513	0.5	11.8
VP	Muscle	25	0.150 ± 0.0625	0.01–1.01	0.1	20
	Kidney		0.144 ± 0.0807	0.01–1.834	0.5	12
OB	Muscle	27	0.026 ± 0.0581	0.001–0.137	0.1	3.7
	Kidney		0.107 ± 0.0593	0.001–1.63	0.5	3.7
All	Muscle	169	0.065 ± 0.0117	0.001–1.01	0.1	13.6
	Kidney		0.183 ± 0.0316	0.001–3.89	0.5	8.9

SM Sisak-Moslavina, *BP* Brod-Posavina, *VS* Vukovar-Srijem, *BB* Bjelovar-Bilogora, *PS* Požega-Slavonia, *VP* Virovitica-Podravina, *OB* Osijek-Baranja

were 2.6 times higher than those measured in animals from Spain (Santiago et al. 1998) or more than tenfold those in Slovakia (Kottferová and Koréneková 1998), though 1.2 to 4 times less than previous findings in Croatia (Bilandžić et al. 2009).

The study indicates regional differences, with renal Cd levels in Bjelovar-Bilogora County significantly lower than in the six other counties ($p < 0.05$ to 0.0001), and in Osijek-Baranja County in comparison with Brod-Posavina and Virovitica-Podravina Counties ($p < 0.05$ to 0.001).

Table 3 Mercury concentrations (mg/kg) in the muscle and kidney of wild boars from seven districts of Croatia

County	Matrix	Number of animals	Mean ± SE	Min–max	Permitted values	Samples exceeding permitted values (%)
SM	Muscle	31	0.006 ± 0.0012	0.001–0.029	0.03	0
	Kidney		0.055 ± 0.0121	0.019–0.229	0.1	9.7
BP	Muscle	20	0.012 ± 0.0029	0.001–0.061	0.03	5
	Kidney		0.152 ± 0.0465	0.001–0.984	0.1	55
VS	Muscle	26	0.01 ± 0.0014	0.002–0.028	0.03	0
	Kidney		0.087 ± 0.0121	0.001–0.277	0.1	26.9
BB	Muscle	23	0.005 ± 0.0011	0.001–0.02	0.03	0
	Kidney		0.040 ± 0.0061	0.006–0.126	0.1	4.3
PS	Muscle	17	0.004 ± 0.0003	0.001–0.007	0.03	0
	Kidney		0.1 ± 0.0141	0.026–0.214	0.1	35.3
VP	Muscle	25	0.009 ± 0.0022	0.001–0.04	0.03	4
	Kidney		0.062 ± 0.0213	0.001–0.545	0.1	8
OB	Muscle	27	0.008 ± 0.0014	0.002–0.036	0.03	3.7
	Kidney		0.066 ± 0.0107	0.003–0.24	0.1	11.5
All	Muscle	169	0.008 ± 0.0014	0.001–0.036	0.03	2.4
	Kidney		0.077 ± 0.0075	0.001–0.984	0.1	19.5

SM Sisak-Moslavina, *BP* Brod-Posavina, *VS* Vukovar-Srijem, *BB* Bjelovar-Bilogora, *PS* Požega-Slavonia, *VP* Virovitica-Podravina, *OB* Osijek-Baranja

However, Cd concentrations in muscle tissue from Virovitica-Podravina County were significantly higher than those in Sisak-Moslavina, Brod-Posavina, Požega-Slavonia, Bjelovar-Bilogora and Osijek-Baranja Counties ($p < 0.05$ to 0.0001). Also, muscle Cd levels from Sisak-Moslavina County were significantly lower than those from Bjelovar-Bilogora, Požega-Slavonia and Vukovar-Srijem Counties ($p < 0.01$, all).

Regulatory agencies in Croatia have established the maximal concentrations permitted according to the Croatian regulations (Official Gazette 16/05) for Cd, Pb and Hg in the meat and kidney (mg/kg) of animals intended for human consumption: 0.1 and 0.5 for Pb, respectively; 0.05 and 1 for Cd; 0.03 and 0.1 for Hg. In this study, muscle Cd levels exceeding the maximum allowed concentration in more than 10% of measured samples were recorded in wild boar from Požega-Slavonia (12.5%), Vukovar-Srijem (23.1%), Bjelovar-Bilogora (17.4%) and Virovitica-Podravina (36%) Counties. In all counties, with the exception of Virovitica-Podravina, renal Cd concentrations exceeding the maximum allowed levels were found in 55.6% to 90% of samples. According to the high percentages of unsuitable samples, wild boars of Vukovar-Srijem and Virovitica-Podravina Counties are highly contaminated with Cd.

In all seven counties, Pb concentrations in muscle were three to ten times lower than those measured in Poland (Kottferová and Koréneková 1998). However, renal Pb levels were 1.6 to 10 times lower than levels found in Spain (Santiago et al. 1998) and 1.5 to 3 times lower than those in Poland (Kottferová and Koréneková 1998), but similar to previous findings in earlier years in Croatia (Bilandžić et al. 2009). The mean concentrations of Pb in the kidney of wild boars from Sisak-Moslavina and Bjelovar-Bilogora Counties were significantly higher than those found in muscle tissues ($p < 0.01$, both). In the remaining five counties, there were no significant differences in Pb levels between tissues. Renal Pb levels in wild boars from Sisak-Moslavina County were significantly higher than those in Brod-Posavina, Osijek-Baranja and Vukovar-Srijem Counties ($p < 0.01$ to 0.001). Also, muscle Pb concentrations of animals from Sisak-Moslavina County were significantly higher than levels in wild boars in Brod-Posavina and Osijek-Baranja Counties ($p < 0.05$ and $p < 0.01$). Also, muscle Pb levels found in Virovitica-Podravina County were above the permitted concentration and significantly higher than levels found in Osijek-Baranja County ($p < 0.05$).

Muscle Pb levels exceeding the maximum allowed concentrations in more than 10% of total samples were recorded in Sisak-Moslavina (32.3%), Vukovar-Srijem (11.5%), Bjelovar-Bilogora (13%) and Virovitica-Podravina (20%) Counties. Pb levels above maximum allowed concentrations in kidney samples measured in more than 10% samples were found in Sisak-Moslavina, Virovitica-Podravina and Požega-Slavonia Counties. According to the obtained percentages of unsuitable samples in muscle and renal tissue of animals, the highest contamination of animals with Pb was found in Sisak-Moslavina and Virovitica-Podravina Counties.

Mean Hg levels in the kidney of wild boars found in all seven counties were significantly higher than those found in muscle tissues ($p < 0.01$ to 0.0001). Average Hg levels in kidney and muscle tissues were consistent with previous findings in the kidney of wild boars in Poland (Dobrowalska and Melosik 2002). In tissues of animals from Brod-Posavina County, mean Hg concentrations were above the permitted concentration in muscle and kidney. Therefore, Hg levels measured in this county are significantly higher than those found in muscle tissue in Sisak-Moslavina and Bjelovar-Bilogora ($p < 0.05$, both) Counties, and in kidney levels in Sisak-Moslavina, Bjelovar-Bilogora and Osijek-Baranja Counties ($p < 0.05$ to 0.01). Low Hg levels were determined in muscle tissues of animals from Požega-Slavonia County, and these were significantly lower than those found in muscle tissues in Brod-Posavina, Osijek-Baranja and Vukovar-Srijem Counties ($p < 0.05$ to 0.001). However, Hg kidney concentrations in Požega-Slavonia County were significantly higher than those found in Sisak-Moslavina and Bjelovar-Bilogora Counties ($p < 0.01$ and $p < 0.0001$). As previously mentioned, the highest Hg concentrations in kidneys were measured in Brod-Posavina County, i.e. renal tissue concentrations exceeding the permitted values were found in 55% of samples. Values exceeding the permitted limits in renal tissue in more than 10% of samples were found in Vukovar-Srijem (26.9%), Požega-Slavonia (35.3%) and Osijek-Baranja (11.5%) Counties.

Taking all values from the seven regions into account, muscle and renal tissues exceeded the permitted values as follows: for Cd 13.6% and 71.6% of samples, respectively; and for Pb 13.6% and 8.9%. Also it has been determined that only 2.9% of samples had values exceeding 1 mg/kg of Pb levels, which is 4.1 times lower, whereas Cd values exceeded the levels found in Spain by 1.5 times (Santiago et al. 1998). However, in all seven counties together, 19.5% renal samples are not suitable for use due to concentrations exceeding the permitted Hg values. Elevated concentration of Hg above the permitted levels was also found in the kidney of wild boars (Dobrowalska and Melosik 2002).

None of the seven studied areas are known for mining activities, industrial production or pollution sources that would cause the accumulation of Cd, Pb and Hg and contribute to contamination of tissues in free-living animals, as recorded in European countries such as Slovenia (Doganoc and Šinigoj-Gačnik 1995; Pokorny and Ribarič-Lasnik 2000), Spain (Santiago et al. 1998; Reglero et al. 2008), Germany (Kierdorf and Kierdorf 2002) and Poland (Dobrowalska and Melosik 2002; Falandysz et al. 2005). 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 the day, thus 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 (Satarug et al. 2003; Reglero et al. 2008).

In summary, we have shown that wild boars, as free-migrating animals on the hunting grounds of the continental Croatia, are highly contaminated with Cd and Pb. Particularly worrying is the presence of Hg in renal tissue. These results indicate the need to discover the sources of animal contamination and to conduct intensified control of meat and elimination of organ meats from human nutrition. Further studies should address the sources of contamination for wild boar and different factors such as age, seasonal variations, food preferences and diet composition.

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