THE INTERNATIONAL SYMPOSIUM ON ANIMAL SCIENCE (ISAS) 2015
19th INTERNATIONAL CONGRESS ON BIOTECHNOLOGY IN ANIMAL REPRODUCTION (ICBAR)

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LEAD AND CADMIUM IN WILD BOAR FROM DIFFERENT GROUNDS IN SERBIA

Živkov Baloš M.¹, Mihaljev Ž.¹, Ljubojević D.¹, Apić J.¹

Abstract: Levels of lead (Pb) and cadmium (Cd) in the liver and kidney of 41 individual wild boars, hunted in 16 Serbian regions, were determined. The samples were prepared by wet digestion using Ethos, Labstation Microwave, Milestone. Content of investigated elements was determined by the method of coupled plasma on the Agilent ICP-MS 7700. Pb and Cd levels in liver and kidney were within the following range (ppm): liver Pb - 0.303±0.219; Cd - 0.295±0.277; kidney Pb – 0.299±0.246; Cd –2.130±1.840. Results from this study showed that Cd concentration in the kidney of wild boar were higher than those found in the liver. The findings showed renal and liver Pb and Cd differences between different grounds. Because of the high Cd level in the organs of wild boar, further research is needed to identify the source of contamination in order to preserve the health of humans and animals.

Keywords: lead, cadmium, liver, kidney, wild boar

Introduction

Various anthropogenic emission activities are a major cause of increased environmental concentrations of cadmium (Cd) and lead (Pb). Since there is no effective mechanism for toxic element elimination due to their potential toxicity and accumulation in various compartments, chronic lower level intakes have damaging effects on both human and animals (Bilandžić et al., 2010). The actual risk presented by toxic elements to wildlife and humans is mostly demonstrated as a chronic sub-lethal effect (immunopathology, teratogenicity, carcinogenicity, and changes in the reproductive system) and requires the implementation of pollution monitoring procedures (Yarsan et al., 2014).

Many wild animals are exposed to different toxic substances by consuming contaminated plants and animals, or water, soil and air. 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). Because animals can move freely and find their own food, the game is a link in the chain that accumulates pollutants from the environment. Variations in mineral matter (metals and non-metals) content in plants are due to a variety of factors, including plant species, plant age, pedological features of soil, climate and implementation of agrotechnical measures. Toxic elements analyses of soils, feed (alfalfa and maize) and food (wheat) indicated a high variability in the contents of these elements between some locations in Vojvodina Province (Živkov-Baloš et al., 2000, 2011). The same plant species differ in microelement content under different ecological

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conditions, while diverse species in the same biotope accumulate different amounts of microelements (Mihaljev et al., 2014). Therefore, wildlife species on certain geographic areas may be a good indicator of pollution, especially of certain chemical elements, as they eat unprocessed plants in a particular habitat. It should be noted that the accumulation of toxic elements is affected by endogenous factors (age, sex, health status of animals) and exogenous factors (geography, hydrological conditions, soil, climate, plant life). Lead (Pb) is a natural constituent of the biogeo sphere 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 organo lead additives to gasoline. Environmental concentrations of cadmium (Cd) are significantly increasing 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 (Satarug et al. 2003; Włostowski et al., 2006). Following oral exposure to Cd in food and drinking water, it is preferentially accumulated in the liver and kidneys of animals (Włostowski et al. 2006: Živković-Baloš et al., 2013).

The purpose of this study was to evaluate the concentration of environmental contaminants Pb and Cd in tissues of free-living wild boars in Serbia, as important information in conducting risk assessments for wildlife and for humans. The results obtained were compared with relevant data reported from other countries.

Material and Methods

Samples of liver and kidney of wild boar (Sus scrofa) shot by hunters were collected from 16 regions in Serbia: Zrenjanin, Novi Kneževac, Novi Sad, Šid, Bač, Bačka Palanka, Apatin, Sombor, Subotica, Sremška Mitrovica, Ruma, Pećinci, Irig, Niš, Bujanovaci Bukevick. Sampling of the wild animal organs was performed during the 2013/2014 hunting season. Animals were not selected according to sex or age. Thus, liver and kidney samples were collected from each animal (total of 41 animals). Upon collection, all samples were placed into labelled plastic bags and stored at -18°C to avoid tissue degradation prior to analysis. The samples were prepared by wet digestion using Ethos, Labstation Microwave, Milestone. The samples were prepared applying the microwave digestion method (14) with the use of the mixture H₂O₂/HNO₃ (1:4, v/v). After this process, the samples were transferred to 50 mL volumetric flasks and diluted with deionized water. Analyses of lead (NoG-M, IT 0.1 s/P) and cadmium (NoG-M, IT 1 s/P) were conducted by Agilent ICP-MS 7700 mass spectrometer by using isotopes ²⁰⁸Pb and ¹¹¹Cd. Limits of quantification (LODs) were 0.001 mg/kg for Pb and 0.001 mg/kg for Cd. Recovery rates for Pb was 88.1% and for Cd 96.1%.

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

Results and Discussion

Concentrations of Pb and Cd in liver and kidney of wild boars are presented in Table 1. We examined total of 64 samples (28 samples of liver and 36 samples of kidney) of 41 wild boars from a total of 16 locations.
Table 1. Lead and cadmium concentrations (mg/kg) in the liver and kidney of wild boars from different localities of Serbia

<table>
<thead>
<tr>
<th>Locality (No of samples)</th>
<th>Liver</th>
<th></th>
<th></th>
<th>Kidney</th>
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<tbody>
<tr>
<td></td>
<td>Pb</td>
<td>Cd</td>
<td>Pb</td>
<td>Cd</td>
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<tr>
<td></td>
<td>Mean±S.E.</td>
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<td>Range</td>
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<tr>
<td>Zrenjanin (2)</td>
<td>n.i.</td>
<td>n.i.</td>
<td>0.166</td>
<td>2.600</td>
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<tr>
<td>Novi Kneževac (3)</td>
<td>0.423±0.023</td>
<td>0.256±0.175</td>
<td>n.i.</td>
<td>n.i.</td>
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<tr>
<td></td>
<td>0.407-0.440</td>
<td>0.133-0.380</td>
<td></td>
<td></td>
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<tr>
<td>Novi Sad (2)</td>
<td>0.904</td>
<td>0.234</td>
<td>0.902</td>
<td>4.063</td>
<td></td>
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</tr>
<tr>
<td>Bač (12)</td>
<td>0.160±0.141</td>
<td>0.111±0.044</td>
<td>0.360±0.202</td>
<td>0.452±0.391</td>
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<tr>
<td></td>
<td>0.001-0.326</td>
<td>0.056-0.156</td>
<td>0.198-0.653</td>
<td>0.194-1.034</td>
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<tr>
<td>Šid (4)</td>
<td>0.063±0.009</td>
<td>0.754±0.428</td>
<td>0.098±0.086</td>
<td>6.162±0.176</td>
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<tr>
<td></td>
<td>0.057-0.070</td>
<td>0.452-1.057</td>
<td>0.038-0.159</td>
<td>5.913-6.162</td>
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<tr>
<td>Bačka Palanka (4)</td>
<td>0.434±0.247</td>
<td>0.412±0.375</td>
<td>n.i.</td>
<td>n.i.</td>
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<tr>
<td></td>
<td>0.253-0.715</td>
<td>0.021-0.916</td>
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<tr>
<td>Subotica (1)</td>
<td>0.748</td>
<td>0.852</td>
<td>n.i.</td>
<td>n.i.</td>
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<tr>
<td>Sombor (4)</td>
<td>n.i.</td>
<td>n.i.</td>
<td>0.201±0.014</td>
<td>0.414±0.401</td>
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<td></td>
<td>0.181-0.939</td>
<td>0.07-0.939</td>
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<tr>
<td>Apatin (4)</td>
<td>n.i.</td>
<td>n.i.</td>
<td>0.129±0.034</td>
<td>1.997±0.905</td>
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<td></td>
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<td></td>
<td>0.111-0.180</td>
<td>0.841-3.028</td>
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<tr>
<td>Sremska Mitrovica (4)</td>
<td>0.205±0.035</td>
<td>0.147±0.0474</td>
<td>0.302±0.114</td>
<td>2.706±1.270</td>
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<td></td>
<td>0.180-0.230</td>
<td>0.114-0.181</td>
<td>0.161-0.437</td>
<td>1.647-4.497</td>
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<td>Ruma (3)</td>
<td>n.i.</td>
<td>n.i.</td>
<td>0.318±0.087</td>
<td>1.449±0.257</td>
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<td>0.227-0.400</td>
<td>1.162-1.659</td>
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<tr>
<td>Pećinci (4)</td>
<td>0.196±0.055</td>
<td>0.0610±0.0566</td>
<td>0.182</td>
<td>0.658</td>
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<tr>
<td></td>
<td>0.133-0.236</td>
<td>0.012-0.123</td>
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<tr>
<td>Irig (6)</td>
<td>0.261±0.045</td>
<td>0.380±0.261</td>
<td>0.303±0.081</td>
<td>1.392±0.976</td>
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<td></td>
<td>0.209-0.288</td>
<td>0.138-0.656</td>
<td>0.240-0.394</td>
<td>0.702-2.082</td>
<td></td>
<td></td>
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<tr>
<td>Niš (1)</td>
<td>0.222</td>
<td>0.542</td>
<td>n.i.</td>
<td>n.i.</td>
<td></td>
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</tr>
<tr>
<td>Bujanovac (8)</td>
<td>0.240±0.080</td>
<td>0.1865±0.0573</td>
<td>0.226±0.053</td>
<td>2.641±1.276</td>
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<tr>
<td></td>
<td>0.177-0.364</td>
<td>0.134-0.240</td>
<td>0.159-0.287</td>
<td>1.213-3.958</td>
<td></td>
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</tr>
<tr>
<td>Bukovik (2)</td>
<td>0.646</td>
<td>0.165</td>
<td>1.385</td>
<td>1.28</td>
<td></td>
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</tr>
</tbody>
</table>

n.i. - not investigated

Considering the lack of referent values for the maximum allowed levels (MAL) of metals and non-metals in tissues of wild species, the obtained results were evaluated and compared applying the maximum allowed levels of particular contaminant in food and feed in Republic of Serbia (Official Gazette, 2011).

Maximum allowed level of Pb in liver and kidney (entails of domestic animals) is 0.50 mg/kg. Mean Pb concentrations in liver of wild boars from different localities of Serbia was 0.303±0.219 mg/kg with range 0.0010-0.904 mg/kg. In this study, liver Pb levels that exceeded MAL in more than 10% (14.3%) of measured samples were recorded in animals from Novi Sad, Subotica, Bačka Palanka and Bukovik. Mean Pb concentrations in kidney of wild boars was 0.299±0.246 mg/kg with range 0.038-1.385 mg/kg. The kidney Pb levels that exceeded MAL in 5.5% of measured samples were recorded in animals from Novi Sad and Bukovik. The study indicates the differences in the content of Pb and Cd between
individuals, organs, and territorial differences. The mean liver Pb were slightly higher than kidney Pb, which is also in accordance with the results of other authors (Santiago et al., 1998; Bilandžić et al., 2009; Bilandžić et al., 2010). The measured values of the lead content in liver and kidneys of wild boars from the territory of Serbia were somewhat lower than in the researches of the other authors. Bilandžić et al. (2009) found that the mean liver Pb of wild boars from different regions of Croatia ranged from 0.061-0.202 mg/kg, while the kidney Pb was 0.056-11.60 mg/kg. Santiago et al. (1998) presented that the mean liver Pb content of wild boars from Spain (Andalusia) amounted 2.61 mg/kg (0.11-4.06 mg/kg), and kidney Pb 0.62 (0.10-4.34) mg/kg. Świergosz et al. (1993) in their study found that kidney Pb of wild boars from Poland was 1.4±2.5 mg/kg.

Maximum allowed levels of Cd in liver and kidney (entails of domestic animals) are 0.50 mg/kg and 1.0 mg/kg, respectively. Mean Cd concentrations in liver of wild boars from different localities of Serbia was 0.295±0.2774 mg/kg within range of 0.012-1.057 mg/kg. The liver Cd levels that exceeded MAL in more than 10% (14.3%) of measured samples were recorded in animals from Niš, Šid, Subotica and Bačka Palanka. Mean Cd concentrations in kidney of wild boars was 2.130±1.840 mg/kg within range of 0.07-7.851 mg/kg. The kidney Cd levels that exceeded MAL in 63.9% of measured samples were recorded in animals from Zrenjanin, Novi Kneževac, Novi Sad, Šid, Apatin, Sremjska Mitrovica, Ruma, Irig, Bujanovac and Bukovik. Incidence of renal Cd levels of 1-3 mg/kg was 60.9%, while frequency of renal Cd levels above 3 mg/kg was 39.1%. Results obtained in this study were consistent with reports of other authors. Bilandžić et al. (2009) reported that the content of Cd in liver of wild boars from different regions of Croatia ranged from 0.11-0.49 mg/kg, and that liver Cd concentration that exceeded MAL was recorded in 25.0% and 22.2% of wild boar liver samples from two regions (Virovitica-Podravina and Vukovar-Srem). Simultaneously, the content of Cd in kidneys of wild boars amounted 3.47-21.10 mg/kg. Bilandžić et al. (2010) published that the average content of Cd in kidneys of the test subjects from different regions of Croatia amounted 0.866-4.58 mg/kg. In all seven examined regions, concentrations exceeded MAL in 71.6% of samples. However, in the researches that were conducted by Petrović et al. (2013) the content of Cd in liver of wild boars from different regions of the Republic of Serbia ranged from 0.03 to 0.96 mg/kg, and that 46% of samples did not meet the requirements proposed by Serbian regulation (Official Gazette, 2011). Kidney Cd ranged from 0.48-6.84 mg/kg, and the measured content of Cd in 82% of samples was above MAL. The regions in Serbia where the samples originated from, did not match the ones from our research.

Santiago et al. (1998) in their research about an analytical survey of metallic contamination of wild boar from Spain, presented that the concentration of Cd, that was measured in kidneys, was 0.39-5.48 mg/kg (mean=2.16 mg/kg), and in liver 0.28 mg/kg. Frequency distribution of Cd in wild boar from Southern Spain for incidence of Cd levels in liver below 0.5 mg/kg was 86.2% and 13.8% above MAL. Świergosz et al. (1993) in their research of the amount of selected potentially toxic elements in tissues of wild boars from southern Poland, reported that the average content of Cd in kidneys was 37 mg/kg and 89.8 mg/kg, which is considerably higher in regards to admissible concentrations of Cd in animal products.

Wolkers et al. (1994) and Włostowski et al. (2006) reported that Cd and Pb concentrations were higher in the wild animals compared to the farm animals, especially in the kidney, and that there were differences in the content of toxic elements related to sex and age. However, in our research wild boars were not selected by age and sex, so that is why the results cannot be interpreted in such a way.
Wild boar kidneys and livers (butina slightly smaller percentage) are unsuitable for consumption. However, the consumption rate of wild boar entrails is very low, and is probably only higher in hunters. The results of tests on the content of Pb and Cd in the organs of wild boars from different regions of Serbia indicate that these toxic elements are naturally present in the environment, but that the differences exist in relation with geographic regions. The areas, in which animals are caught, are not burdened with industrial plants and coals. The major metal exposure route for wildlife is ingestion and a minor inhalation component (Petrović et al., 2013). Wild boar is an omnivorous species and lead and cadmium are present in all ecosystems. Environmental exposure to Cd determines a distribution pattern in which the renal levels are much higher than hepatic levels (Santiago et al., 1998; Parsons, 1999). In all the animals studied, the renal Cd were much higher than liver Cd, and so it could be concluded that they had been exposed chronically to environmental cadmium.

**Conclusion**

The accumulation of toxic substances in the tissues of game animals is studied almost world-wide. Data from this study may be part of a base to evaluate the levels and trends in contamination of tissues of wild boars in Serbia. Further work is needed to investigate the source of contamination in order to preserve the health of humans and animals.

**References**


88