Distribution of cadmium in leg muscle and liver of game birds from Serbia

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Abstract. The aim of this study was to present the distribution of cadmium (Cd) levels in leg muscle and liver of game birds. Samples (n=464) of: pheasants (n=182), mallards (n=25), Eurasian jay (n=7), partridges (n=5), woodcocks (n=8) and common quail (n=5) were collected during regular hunting seasons within the Serbian National Residue Monitoring Program from 2013 to 2016. Analysis of Cd was performed by ICP-MS. In all liver samples, Cd levels were above the limit of detection (LOD=0.001 mg/kg) while in 66.4% of muscle samples, Cd was detected. Statistical analysis showed significant differences between Cd levels in leg muscle and liver of woodcocks and others game birds. The highest mean Cd level was observed in muscle samples of woodcocks (0.042 mg/kg). The lowest mean Cd levels in liver were observed in common quails (0.130 mg/kg) and mallards (0.160 mg/kg) while the highest levels were measured in woodcocks (1.247 mg/kg) and pheasants (0.262 mg/kg). During four years of the Serbian National Residue Monitoring Program, leg muscle samples of woodcocks (n=3), liver samples of pheasants (n=23), woodcocks (n=6) and mallards (n=3) exceeded the maximum residue limit (MRL).

1. Introduction
Environmental pollution with heavy metals is world-wide problem [1]. Human activities, technological and industrial development all influence heavy metal contamination in the environment. Cadmium (Cd) occurs on agricultural land as a contaminant of phosphorous fertilizers and can be found in sewage sludge, surface waters and plants. Cd is a ubiquitous and toxic heavy metal, recognized as a potential health threat to human and wildlife species as it is not biodegradable and accumulates in living organisms [2, 3]. According to the International Agency for Research on Cancer, Cd and its compounds are classified as carcinogenic to humans [4].

Game and game birds are free-living wildlife. As they freely choose food and are part of soil-plant-animal chain, Cd contamination in free-living game could be a fair bioindicator of environmental pollution [5]. Accumulation of Cd in tissues of game, including game birds is studied almost worldwide [6-9].

Scientific information about Cd levels in tissue of game birds from Serbia is scarce. The aim of this study was to determine and compare distribution of Cd in leg muscle and liver of different species of game birds.
2. Materials and Methods

Levels of Cd were measured in leg muscle and liver of game birds (n=464). Pheasants (n=182), mallards (n=25), Eurasian jay (n=7), partridges (n=5), woodcocks (n=8) and common quail (n=5) were acquired during regular hunting seasons and analysed within the Serbian National Residue Monitoring Program from 2013 to 2016.

Frozen samples were thawed at 4°C the day before the analysis and then homogenized. An amount of approximately 0.5 g of homogenized tissue was transferred into a teflon vessel with 5 mL of nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and 1.5 mL of hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MA, USA) for microwave digestion. The microwave oven (Start D, Milestone, Sorisole, Italy) program consisted of three steps: 5 min from room temperature to 180°C, 10 min hold at 180°C and 20 min ventilation. After cooling, the digested sample solutions were quantitatively transferred into polypropylene volumetric flasks and diluted to 100 mL with deionized water obtained from a water purification system (Purelab DV35, ELGA, Buckinghamshire, UK).

Inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany), was used for analysis of the 111Cd isotope. A five-point calibration curve (including zero) was constructed for the qualitative analysis of the samples. Multielement internal standard (6Li, 45Sc—10 ng/mL; 71Ga, 89Y, 209Bi—2 ng/mL) was introduced online by an additional line through the peristaltic pump.

The quality of the analytical process was verified by analysis of the certified reference material NIST 1577c (Gaithersburg, MD, USA). Reference material was prepared in the same way as samples using microwave digestion. Obtained concentrations were in the range of certified values.

Statistical analysis of experimental data was performed using Minitab 16 Statistical software. One-way analysis of variance – ANOVA and Tukey’s HSD test were applied for comparison of Cd levels between different leg muscles samples as well as between livers from different game birds. For the purpose of calculation, when the levels of Cd were below the limit of detection (LOD, LOD=0.001 mg/kg), that value was assumed to be equal to 1/2 LOD.

3. Results and Discussion

The results of Cd levels in leg muscle and liver of the analysed samples are presented in table 1 and table 2, respectively.

| Table 1. Cd levels in leg muscle of game birds from Serbia. |
|-----------------|---------|------------------|-----------------|
|                 | n1      | min-max          | Mean ± SD       |
| Pheasants       | 182     | < LOD-0.049      | 0.006±0.008\textsuperscript{a}|
| Mallards        | 25      | < LOD-0.026      | 0.005±0.006\textsuperscript{a}|
| Eurasian jay    | 7       | 0.003-0.010      | 0.005±0.003\textsuperscript{a}|
| Partridges      | 5       | < LOD-0.008      | 0.004±0.003\textsuperscript{a}|
| Woodcocks       | 8       | 0.018-0.065      | 0.042±0.019\textsuperscript{b}|
| Common quail    | 5       | < LOD-0.028      | 0.008±0.012\textsuperscript{a}|

n1 – number of samples,
n2 – number of non-compliant samples.
\textsuperscript{a,b} Different superscripts within the same column indicate significant differences of means according to Tukey’s HSD test (p < 0.05).
Table 2. Cd levels in liver of game birds from Serbia.

|              | n1  | min-max       | Mean ± SD       | n2  |
|--------------|-----|---------------|-----------------|-----|
| Pheasants    | 182 | 0.014-1.162   | 0.262±0.257\textsuperscript{a} | 23  |
| Mallards     | 25  | 0.005-0.746   | 0.186±0.166\textsuperscript{a} | 2   |
| Eurasian jay | 7   | 0.084-0.277   | 0.174±0.086\textsuperscript{a} |     |
| Partridges   | 5   | 0.084-0.292   | 0.160±0.078\textsuperscript{a} |     |
| Woodcocks    | 8   | 0.213-3.204   | 1.247±0.989\textsuperscript{b} | 6   |
| Common quail | 5   | 0.005-0.467   | 0.130±0.190\textsuperscript{a} |     |

n1 – number of samples.
n2 – number of non-compliant samples.
\textsuperscript{a,b} Different superscripts within the same column indicate significant differences of means according to Tukey’s HSD test (p < 0.05).

The levels of Cd in leg muscle were from < 0.001 to 0.042 mg/kg. The highest percentages of detected Cd levels in leg muscle were detected in woodcocks, partridges and common quails (figure 1). The highest mean Cd level was observed in leg muscle of woodcocks (0.042 mg/kg) while in other game birds, Cd was found only at very low levels, often close to the LOD (table 1). Statistical analysis showed significant differences between Cd levels in leg muscle of woodcocks and others game birds. According to the national legislation [10] there is no maximum residue level (MRL) for Cd in game tissue, therefore MRL for Cd in poultry tissue (muscle, liver) was used instead. The MRL for Cd in poultry muscle is 0.050 mg/kg [10]. Three leg muscle samples of woodcocks analysed during four years (2013-2016) exceeded this level i.e. those samples were non-compliant.

![Figure 1. Percentage (%) of leg muscle samples of game birds with detected Cd levels.](image)

While Cd levels in leg muscle was mostly low, liver accumulated higher levels [8, 11, 12] resulting in detectable levels of Cd in all liver samples examined. The lowest mean Cd levels in liver were established in livers from common quail (0.130 mg/kg) and mallards (0.160 mg/kg) while the highest were in livers from woodcocks (1.247 mg/kg) and pheasants (0.262 mg/kg). Significant differences were established between Cd levels in liver of woodcocks and others game birds. National legislation [10] established 0.500 mg/kg as the MRL for Cd in game liver. Some livers of pheasants (n=23), mallards (n=2) and woodcocks (n=6) exceeded this level. The highest measured Cd levels were in liver of mallard (3.204 mg/kg) and pheasant (1.162 mg/kg).

To the best of our knowledge, most of the literature on the subject of Cd levels in tissue of game birds is for pheasants and mallards. According to Petrovic and Jankovic [9], two liver samples (0.70
and 0.95 mg/kg) of pheasants collected during hunting seasons 2004 and 2005 in Serbia exceeded the MRL. The mean Cd level in leg muscle of pheasants from the current study was lower while in liver, it was higher compared to the mean Cd levels reported by Korénková et al. [13] (0.019 and 0.024 mg/kg, respectively). Szymczyk and Zalewski [14] reported lower mean Cd level in pheasants’ livers (range from 0.130 to 0.180 mg/kg) in birds living near a non-ferrous metallurgy area as well as Cd levels among their non-compliant samples (1.121 mg/kg) than we obtained in this study. Also, Cd levels in liver of mallards were examined in Szymczyk’s and Zalewski’s [14] study. The authors reported Cd levels in liver of analysed mallards in the range of 0.014 to 0.394 mg/kg, which was lower compared to our data, while higher levels of Cd in liver were found in mallards from the polluted region of Ślask (average 1.274 mg/kg) [14].

4. Conclusion

During the Serbian National Residue Monitoring Program from 2013 to 2016, 7% of leg muscle and liver samples of game birds were non-compliant for Cd levels. Hunters and members of their households commonly eat game meat, thus intaking higher amounts of Cd than the average population. It is assumed that the amount of consumed game meat could pose a health risk even in this small part of the population. Therefore, continual control of Cd and other toxic elements in game meat is needed in order to assess the safety of these tissues with respect to human health.

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