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Cadmium in pheasant tissues as a bioindicator of environmental pollution in 23 Serbian districts

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A b s t r a c t: Concentrations of cadmium (Cd) were measured in leg muscle and liver of pheasants (n=316) from 23 different Serbian regions under the Serbian National Residue Monitoring Program during 2013-2015. Levels of Cd in pheasant tissues were determined by inductively-coupled plasma mass spectrometry (ICP-MS). Most of the pheasants examined had Cd in their tissues. In the third year of the residue monitoring program, the Cd concentrations detected in leg muscles were nearly double the levels detected in the first year. The highest Cd levels in muscles were measured in pheasants from Podunavlje, Macva and Zlatibor districts. All leg muscle samples had Cd concentrations below permitted maximum residue levels (MRL=0.05 m kg^{-1}). However, fifteen liver samples had Cd levels that exceeded the permitted MRL (0.50 mg kg^{-1}). The number of non-compliant pheasant liver samples increased over the years. The lowest mean Cd level in pheasant livers was measured in birds from Morava district (0.035 mg kg^{-1}), while the highest was in birds from Branicevo district (0.574 mg kg^{-1}). This monitoring program shows that the Cd levels measured in pheasants, which are suitable bioindicators of environmental pollution, indicate that environmental pollution with Cd is increasing.

Keywords: *pheasants, cadmium, leg muscle, liver, Serbian districts.*

Introduction

Industrial development has played an important role in environmental pollution, including in heavy metal toxicity in the biosphere, leading to severe environmental and health hazards. Different heavy metals are found in the environment as natural components. However, heavy metals primarily get into foods of animal origin and the bodies of human consumers due to anthropogenic (industrial, agricultural, traffic) activities (*Tchounwou et al*., 2012). Heavy metals are not biodegradable, but are accumulated in living organisms and metabolised mostly to more toxic, rarely to less toxic derivatives by biochemical processes (*Lehel et al*., 2016). Cadmium (Cd) is one of the most toxic heavy metals and poses a significant health risk to humans (*Järup et al*., 1998). Pollution of the environment and contamination of animals with Cd is a problem in most countries (*Hecht et al*., 1984; *Stawarz et al*., 2003). When released into the atmosphere by smelting, mining or some other processes, Cd compounds can be associated with respirable-sized airborne particles and can be carried long distances. It moves easily through soil layers and enters the food chain via plant uptake (*Alexander et al.,* 2009**)**. Cd is also found in meat, especially offal such as liver and kidney (*MacLachlan et al*., 2016; *Ertl et al*., 2016; *Wua et al*., 2016). According to the US poison and disease registry (*Priority List of Hazardous Substances*, 2015), Cd ranks seventh among toxic substances for human health hazard. The International Agency for Research on Cancer (*IARC,* 2012) declared Cd and Cd compounds carcinogenic to humans.

Game, including game birds, as a representative of wildlife, is considered as a suitable bioindicator of potential environmental pollution with Cd (*Mochizuki et al.,* 2002). Levels of Cd in domestic animals and game are generally very different, because game species have the freedom to choose their food. Their diet depends on the seasonal availability of certain types of food and they feed over a wide area and mainly live longer than domestic animals, whose nutrition is uniform and controlled, and thus, livestock have lower levels of Cd (*Kramárová et al.,* 2005; *Toman et al.,* 2005). The accumulation of toxic substances in the tissues of game birds is studied almost world-wide (*Kramárová et al*., 2005; *Toman et al.,* 2005; *Mochizuki et al.,* 2002; *Petrovic and Jankovic*, 2008). *Lazarus et al.* (2014) reported a

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high percentage of free-living game liver and kidney samples exceeding the Croatian national legislative limits for Cd and lead, and recommended that people should limit consumption of offal from certain game species. Also, children, pregnant and lactating women should avoid eating game offal altogether.

Serbia has a long hunting tradition with a large number of well-kept hunting grounds, where pheasants are among the most popular species hunted. According to the *Statistical Yearbook of the Republic of Serbia* (2015), 146,000 pheasants were shot during 2013. The aim of this study was to establish the distribution of Cd in pheasant tissues, as a suitable bioindicator of environmental pollution, from Serbian districts.

Materials and Methods

Concentrations of Cd were measured in leg muscle and liver of pheasants (n=316) from different Serbian regions within the Serbian National Residue Monitoring Program in 2013 (n=134), 2014 (n=98) and 2015 (n=84). The pheasants were acquired from 23 of the 29 districts in Serbia during regular hunting seasons (Figure 1). Pheasants were chosen for this biological monitoring campaign based on the specificity of the districts and their availability in every specific location.

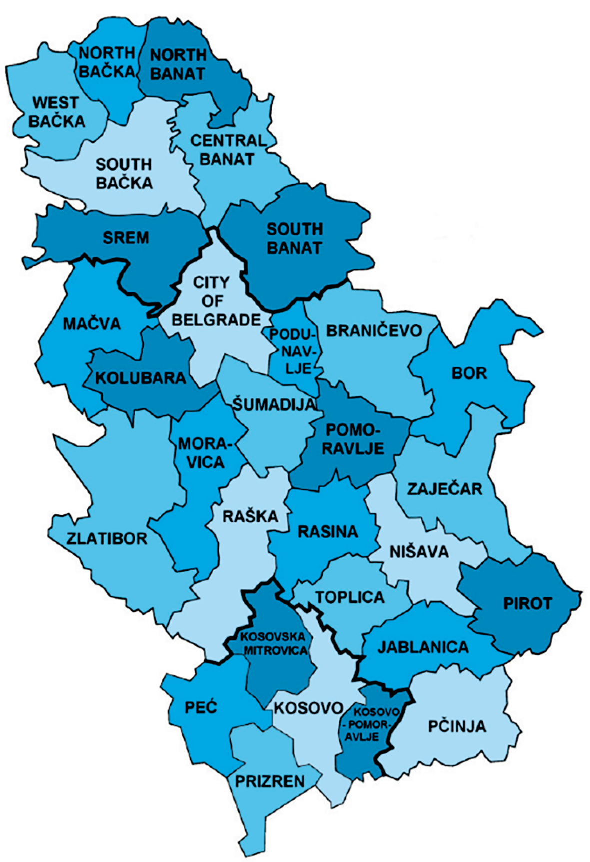

Figure 1. Map of 29 Serbian districts

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

Analysis of the ^{111}Cd isotope was performed by inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). For quantitative analysis of the samples, a five-point calibration curve (including zero) was constructed for Cd isotope. Multielement internal standard (^{6}Li, ^{45}Sc at 10 ng mL^{-1}; ^{71}Ga, ^{89}Y, ^{209}Bi at 2 ng mL^{-1}) 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 manner as the samples, using microwave digestion. Replicate analyses were in the range of certified values.

Statistical analysis of data was performed using software Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). One way analysis of variance – ANOVA – and Tukey's HSD test were used to compare Cd concentrations between leg muscles as well as between livers from the different Serbian districts. Differences were considered significant if $p<0.05$.

Results and Discussion

The Cd concentrations measured in leg muscle and liver of the examined pheasants are summarised in Tables 1 and 2, respectively. For calculation, when the concentration of Cd was below the limit of detection (LOD, LOD=0.001 mg kg^{-1}), that value was assumed to be equal to one half of the LOD (1/2 LOD).

Table 1. Concentration (mg kg^{-1}) of cadmium in pheasant leg muscle by district

District	n[a]	Min-max	(Mean±SD)[b]
South Backa	4	< LOD-0.007	0.004±0.002
Belgrade	14	0.009-0.018	0.008±0.006
South Banat	5	< LOD-0.018	0.007±0.006
Rasina	7	< LOD-0.008	0.003±0.002
Sumadija	9	0.002-0.010	0.005±0.003
Morava	6	< LOD-0.004	0.002±0.001
Kolubara	9	< LOD-0.017	0.008±0.005
Nis	9	< LOD-0.017	0.005±0.005
North Banat	9	< LOD-0.025	0.007±0.008
Branicevo	3	< LOD-0.012	0.007±0.005
Jablanica	6	< LOD-0.006	0.003±0.002
Bor	6	< LOD-0.013	0.005±0.005
Raska	9	< LOD-0.012	0.005±0.004
Pomoravlje	6	< LOD-0.011	0.003±0.004
Zlatibor	12	0.002-0.037	0.007±0.010
Macva	6	< LOD-0.039	0.008±0.015
Podunavlje	9	< LOD-0.049	0.010±0.016
Zajecar	5	0.002-0.012	0.005±0.004
North Backa	4	< LOD-0.008	0.003±0.003
South Banat	3	0.005-0.009	0.007±0.002
Srem	9	< LOD-0.005	0.002±0.002
West Backa	10	< LOD-0.004	0.001±0.001
West Banat	1	< LOD	< LOD

Legend: [a]n – number of samples; [b](Mean±SD) – mean value ± standard deviation

Table 2. Concentration (mg kg^{-1}) of cadmium in pheasant livers by district

District	n[a]	Min-max	(Mean±SD)[b]	n1[c] (year)
South Backa	4	0.245-0.421	0.374±0.102	
Belgrade	14	0.046-0.839	0.353±0.242	3 (2015)
South Banat	5	0.094-0.370	0.212±0.115	
Rasina	7	0.140-0.329	0.216±0.065	
Sumadija	9	0.076-0.348	0.196±0.102	
Morava	6	0.014-0.082	0.035±0.025	
Kolubara	9	0.092-1.054	0.408±0.278	1 (2014)
Nis	9	0.093-0.246	0.130±0.048	
North Banat	9	0.028-0.335	0.144±0.099	
Branicevo	3	0.106-1.162	0.574±0.538	1 (2015)
Jablanica	6	0.068-0.811	0.287±0.267	1 (2014)
Bor	6	0.113-0.916	0.363± 0.325	2 (2014)
Raska	9	0.042-1.104	0.353±0.373	1 (2013), 1 (2015)
Pomoravlje	6	0.046-0.143	0.101±0.036	
Zlatibor	12	0.060-0.998	0.291±0.307	1 (2013), 1 (2015)
Macva	6	0.087-0.391	0.217±0.110	
Podunavlje	9	0.204-0.865	0.404±0.236	1 (2014), 2 (2015)
Zajecar	5	0.022-0.170	0.105±0.061	
North Backa	4	0.078-0.248	0.127±0.082	
South Banat	3	0.209-0.462	0.366±0.137	
Srem	9	0.053-0.373	0.168±0.096	
West Backa	10	0.036-0.187	0.091±0.043	
West Banat	1		0.059	

Legend: [a]n – number of samples; [b](Mean±SD) – mean value ± standard deviation; [c]n1– number of non-compliant samples

The concentration of Cd in leg muscles was within the range <0.00–0.049 mg kg^{-1}. During our monitoring, the number of muscle samples in which Cd was detected increased. In 2013, 2014 and 2015, Cd was detected in 43.28%, 65.31% and 83.33% of all pheasant leg muscle samples, respectively. The highest Cd levels in muscles were measured in pheasants from Podunavlje, Macva and Zlatibor districts (Table 1). However, there were no statistically significant differences in Cd concentrations in pheasant leg muscles between different districts. All leg muscles from pheasants sampled in this study had Cd concentrations below maximum residue levels (MRL; 0.050 mg kg^{-1}) for Cd in game muscle according to national legislation (*Serbia*, 2014). Mean Cd concentrations in pheasant leg muscle from all districts (0.005 mg kg^{-1}) determined in this study were lower than those in shot pheasants (0.019 mg kg^{-1}) reported by *Koréneková et al.* (2008).

While Cd concentrations in muscles are generally low, liver and kidney accumulate higher concentrations (*Massányi et al.,* 1995; *Massányi and Uhrín*, 1996; *Toman et al.,* 2005). In the current study, the mean concentration of Cd in pheasant leg muscle (0.005 mg kg^{-1}) was significantly lower than in liver (0.254 mg kg^{-1}). The lowest mean Cd concentration in pheasant liver was measured in birds from the Morava district (0.035 mg kg^{-1}) while the highest concentrations were detected in Branicevo district birds (0.574 mg kg^{-1}). Nevertheless, there were no significant differences in Cd concentrations in pheasant livers from the different districts. According to national legislation (*Serbia*, 2014), the MRL for Cd in game liver is 0.500 mg kg^{-1}. Fifteen pheasant liver samples analysed during three years (2013–2015) exceeded this level, and so were non-compliant (Table 2). The highest Cd levels detected were 1.162, 1.054 and 1.104 mg kg^{-1} in pheasant livers from Branicevo, Kolubara and Raska districts, respectively. The number of non-compliant pheasant liver samples increased over the years. In 2013, only two non-compliant livers were detected, from Raska and Zlatibor districts. The next year, we found five non-compliant pheasant livers from four different districts (two from Bor, and one each from Kolubara, Branicevo and Jablanica), which was 10.2% of the non-compliant samples of all game in 2014. In 2015, 19.05% of the pheasant livers examined were non-compliant (8 non-compliant livers: three from Belgrade, two from Podunavlje and one each from Branicevo, Raska and Zlatibor). *Petrovic and Jankovic* (2008) detected Cd in 21% of all examined liver samples and 2% of them were non-compliant samples originating from Kolubara. The authors explained that the Kolubara basin and the thermal power plants located there may be the reason for high Cd concentrations in pheasant livers from this district. The mean Cd levels in fowl liver samples from birds living in a non-ferrous metallurgy area reported by *Szymczyk and Zalewski* (2003) ranged from 0.13 mg kg^{-1} to 0.18 mg kg^{-1}. These Cd concentrations were lower than those measured in pheasant livers from most Serbian districts in the current study. However, the mean Cd concentration of non-compliant livers measured by *Szymczyk and Zalewski* (2003) (1.12 mg kg^{-1}), was higher than that determined in our study (0.837 mg kg^{-1}). *Koréneková et al.* (2008) established a lower mean Cd level in shot pheasant livers (0.037 mg kg^{-1}) than in our current study (0.254 mg kg^{-1}).

Conclusion

In summary, most of the pheasants examined within the Serbian National Residue Monitoring Program during three years (2013–2015) contained Cd in their tissues. Leg muscles had lower Cd concentrations than livers. In 2013, 2014 and 2015, Cd was detected in 43.28%, 65.31% and 83.33% of all pheasant leg muscle samples, respectively. The highest Cd levels in leg muscles were measured in pheasants from Podunavlje, Macva and Zlatibor districts. All leg muscle samples in this study had Cd concentrations below the permitted MRL. However, the livers of 15 of the pheasants analysed had Cd levels which exceeded the permitted MRL (MRL=0.500 mg kg^{-1}). The number of non-compliant pheasant liver samples increased over the years. The lowest mean Cd level in pheasant livers was measured in birds from Morava district (0.035 mg kg^{-1}), while the highest level was in birds from Branicevo district (0.574 mg kg^{-1}). According to the results from this study and having in mind the small number of samples collected from some districts, it was impossible to conclude which districts likely had the highest environmental Cd pollution. On the other hand, considering the fact that pheasants are suitable bioindicators of environmental pollution, it could be concluded that environmental Cd pollution is increasing. Therefore, levels of Cd should be followed by continued monitoring of pheasant tissues.

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References

Alexander, J., Benford, D., Cockburn, A., Cravedi, J., Dogliotti, E. & Domenico, A. (2009). Cadmium in food – scientific opinion of the panel on contaminants in food chain. *EFSA Journal, (980)*, 1−39.

Ertl, K., Kitzer, R. & Goessler, W. (2016). Elemental composition of game meat from Austria. *Food Additives & Contaminants – Part B, 9 (2*), 120−126.

Hecht, H., Schinner, W. & Kreutzer, W. (1984). Endogene und exagene Einflusse auf die Gehalte an Blei und Cadmium in Muskel und Organproben. 1 Mitteilung: Einfluss von Alter und Versuchsort. *Fleischwirtsch, 64,* 967−969.

IARC, International Agency for Research on Cancer. (2012). IARC Monographs on the evaluation of carcinogenic risks to humans. Arsenic, metals, fibres and dusts. Volume 100 C. A review of human carcinogens. Lyon, France.

Järup, L., Bellander, T., Hogstedt, C., Spang, G. (1998). Mortality and cancer incidence in Swedish battery workers exposed to cadmium and nickel. *Occupational and Environmental Medicine, 55*, 755−759.

Koréneková, B., Skalická, M., Kozárová, I., Nagy, J., Máté, D. & Nad, P. (2008). Comparison of cadmium, lead and nickel accumulation in liver, breast and leg muscles of pheasants. Slovak Journal of Animal Science, 41 *(4)*, 184–186.

Kramárová, M., Massányi, P., Jancová, A., Toman, R., Slamecka, J., Tataruch, F., Kovácik, J., Gasparík, J., Nad, P., Skalická, M., Koréneková, B., Jurcík, R., Cuboň, J. & Hascík, P. (2005). Concentration of cadmium in the liver and kidneys of some wild and farm animals. *Bulletin of the* Veterinary Institute *in* Pulawy, *49,* 465−469.

Lazarus, M., Prevendar Crnic, A., Bilandzic, N., Kusak, J. & Reljic, S. (2014). Cadmium, lead, and mercury exposure assessment among Croatian consumers of free-living game. *Archives of Industrial Hygiene and Toxicology, 65,* 281−292.

Lehel, J., Lanyi, K. − Laczay, P. (2016). Food safety significance of heavy metal contamination in foods of animal origin. *Magyar allatorvosok lapja, 138* (2), 99−112.

MacLachlan, D. J., Budd, K., Connolly, J., Derrick, J., Penrose, L. & Tobin, T. (2016). Arsenic, cadmium, cobalt, copper, lead, mercury, molybdenum, selenium and zinc concentrations in liver, kidney and muscle in Australian sheep. *Journal of Food Composition and Analysis, 50,* 97–107.

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Massányi, P., Toman, R., Uhrín, V. & Renon, P. (1995). Distribution of cadmium in selected organs of rabbits after an acute and chronic administration. *Italian Journal of Food Science, 7,* 311−316.

Massányi, P. & Uhrín, V. (1996). Histological changes in the ovaries of rabbits after an administration of cadmium. *Journal of Environmental Science and Health, A32,* 1459−1446.

Mochizuki, M., Hondo, R., Kumon, K., Sasaki, R., Matsuba, H. & Ueda, F. (2002). Cadmium contamination in wild birds as an indicator of environmental pollution. *Environmental Monitoring and Assessment, 73 (3)*, 229−235.

Petrovic, Z. & Jankovic, S. (2008). Pheasant game as bioindicator of cadmium presence in the environment. *Tehnologija Mesa, 49 (1−2),* 36−40.

Priority List of Hazardous Substances. (2015). Agency for Toxic Substances and Disease Registry. www.atsdr.cdc.gov/spl.

Serbia. (2014). Rulebook on maximum residue limits residues of pesticides in food and feed and food and feed which established maximum permissible residual quantities of pesticides. *Official Gazette of the Republic of Serbia*, *29*.

Statistical Yearbook of the Republic of Serbia. (2015). Belgrade, Serbia.

Stawarz, R., Zakrzewski, M., Marencik, A. & Hraska, S. (2003). Heavy-metal concentration in the toad *Bufo bufo* from a region of Mochovce, Slovakia. *Ekologia-Bratislava, 3,* 292−297.

Szymczyk, K. & Zalewski, K. (2003). Copper, Zinc, Lead and Cadmium Content in Liver and Muscles of Mallards (Anas Platyrhychnos) and Other Hunting Fowl Species in Warmia and Mazury in 1999-2000. *Polish Journal of Environmental Studies, 12 (3)*, 381−386.

Tchounwou, P. B., Yedjou C. G., Patolla, A. K. & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology*, 133−164.

Toman, R., Massányi, P., Lukác, N., Ducsay, L. & Golian, J. (2005). Fertility and content of cadmium in pheasant *(Phasianus colchicus)* following cadmium intake in drinking water. *Exotoxicolgy and Environmental Safety, 62,* 112−117.

Wua, Y., Zhanga, H., Liua, G., Zhanga, J., Wang, J., Yuc, Y. & Lua, S. (2016). Concentrations and health risk assessment of trace elements in animal-derived food in southern China. *Chemosphere, 144,* 564–570.