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Common pheasant as a biomonitoring tool for environmental cadmium levels in Serbia

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A b s t r a c t: Contamination of food by heavy metals is a concerning problem in developing countries. Cadmium is one of the toxic elements that is considered as a marker for environmental contamination. The avian ecosystem is suitable for biomonitoring purposes, especially in cases where stationary sources of pollution are present. Pheasant samples (n = 327) were collected during four hunting seasons within the framework of the Serbian National Residue Monitoring Program, from 2018 to 2021. The level of cadmium in the samples was measured using inductively coupled plasma mass spectrometry (ICP-MS). The average cadmium level in analysed liver and leg muscle samples was 0.306 mg kg^{-1} and 0.009 mg kg^{-1} respectively. Cadmium levels ranged between 0.005–4.206 mg kg^{-1} in liver and < 0.001–0.235 in leg muscle. The cadmium level in pheasants in Serbia has slightly increased numerically (not statistically) over the past four years, so the level should continue to be carefully monitored.

Keywords: *cadmium, biomonitoring, common pheasant, ICP-MS.*

Introduction

Cadmium is one of the heavy metals marked as an environmental contaminant. It is a non-essential element for plants, animals and humans. Sources of cadmium in the environment are industrial activity (by far the largest), followed by its natural occurrence (*EFSA, 2012*).

Cadmium is typically a metal of the 20th century. It is mainly used in battery production and for the manufacture of special alloys. It can be released into the environment by burning fossil fuels and waste, or via heavy industry emissions, fertilizer production and agriculture processes (*Bernard, 2008*).

Released cadmium remains on site for decades. It enters the food chain by uptake from plants. Animals and humans are exposed via food, water and air (*Govind and Madhuri, 2014*). After absorption in the small intestine, most of the ingested cadmium is accumulated in the liver and kidneys, where it is bound to the transport protein, metallothionein (*VKM, 2015*). Liver is the principal organ for cadmium metabolism.

If it is accumulated in high amounts in animals, cadmium is responsible for disruptions of essential element metabolism, and damage and dysfunctions of internal organs. The toxicity of cadmium depends on the general state of the animal, exposure time, ingested concentrations, sex, age etc. (*Swiergosz and Kowalska, 2000*). Stationary sources of pollution present in the biotopes of non-migratory wild a nimals (e.g. birds) are considered suitable for biomonitoring purposes. Birds selected for such research need to fulfil several conditions related to easy detection and capture, abundance, well known biology of the species etc. (*Dzugan et al. 2012*).

Common pheasant (*Phasianus colchicus*) is colourful, medium-sized bird, well adapted and abundant in the biotope of Serbia, which makes it a good choice for heavy metal biomonitoring of the Serbian environment. It can be found near rivers, close to crop fields, or at the edges of forests. Considering that it is also a game bird, common pheasant is an adequate model for biomonitoring of heavy metals (*CABI, 2015*).

Pheasant meat is considered a delicacy, and it is also rich in proteins, essential amino acids, minerals and vitamins, with a good fatty acid profile. Wild pheasants, as a rule, have higher levels of toxic metals than do farmed animals. Levels of contamination principally depend on the nutrition profile of the animals (*Lazarus et al., 2014*).

Table 1 shows a brief overview of research on the origin and distribution of cadmium in liver and muscle of pheasants and birds that share habitats and

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lifestyles with them. The influence of nutrition on farms and in the natural environment as well as from the bird habitat itself was taken into consideration for this study.

The aim of this study was to determine the concentration of cadmium from environmental origin in edible tissue (liver and leg muscle) of common pheasant.

Table 1. Level of cadmium in liver and muscle of different bird species

Animal	Sample	Range (mg kg^{-1})	Mean (mg kg^{-1})	Technique	Source
Common pheasant (*Phasianus colchicus*)	Liver	0.026–2.008[a]	0.721[a]	AAS	Dzugan et al. (2012)
Common pheasant (*Phasianus colchicus*)	Leg muscle[w] Leg muscle[f]	/ /	0.012[b] 0.016[b]	ICP-OES	Flis et al. (2020)
Common pheasant (*Phasianus colchicus*) Mallard (*Anas platyrhynchos*)	Leg muscle Leg muscle	0.0194–0.1084[a] 0.0035–0.2110[a]	0.0336[a] 0.0289[a]	GFAAS	Gasparik et al. (2010)
Common pheasant (*Phasianus colchicus*)	Liver Muscle	/ /	0.033[a] 0.003[a]	FAAS	Celechovska et al. (2008)
Common Pochard (*Aythya ferina*) Mallard (*Anas platyrhynchos*)	Liver Male Female Liver Male Female	 0.016–0.280[a] 0.001–0.115[a] 0.108–0.800 [a] 0.083–0.432 [a]	 0.123 0.044 0.418 0.249	FAAS	*Florijancic et al. (2009)*
Pigeon Jay Black coot	Liver Muscle	/ / / / / /	< LOD[a] 0.09[a] 0.07[a] < LOD [a] < LOD [a] < LOD [a]	ICP-MS	*Medunic et al. (2018)*
Pigeon	Liver Urban Rural	 / /	 0.52[b] 0.44[b]	FAAS	Miliaimi et al. (2016)
Mallard (*Anas platyrhynchos*)	Liver	0.25–1.3[b]	0.65	ICP-AES	Mateo and Guitart (2003)
Eurasian Woodcocks (*Scolopax rusticola*)	Liver	/	4.39[b]	AAS	Kim and Oh (2013)
House sparrow (*Passer domesticus*)	Liver Rural Urban	 / /	 0.009[b] 0.016[b]	ICP MS	Kekkonen et al. (2012)
Mallard (*Anas platyrhynchos*)	Liver	0.66–2.03[b]	/	GFAAS	Aloupi et al. (2017)
Common pheasant (*Phasianus colchicus*)	Liver Muscle	0.014–1.162[a] < LOD–0.049[a]	0,262 [a] 0,006 [a]	ICP MS	*Nikolic et al. (2017)*

Legend: [a] – wet weight, [b] – dry weight, [w] – wild, [f] – farm

Materials a nd methods

Cadmium levels were measured in liver and leg muscle of common pheasants in the period of four hunting seasons within the framework of the Serbian National Residue Monitoring Program (from 2018 to 2021). The total number of samples analysed was 327.

Samples were stored at −18°C. Frozen samples were thawed at 4°C one day before the analysis and subsequently homogenized. Approximately 0.3 g of each sample tissue was accurately weighed (± 0.001 g) and transferred into a Teflon vessel of a microwave digestion system with 5 mL 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 (Start D, Milestone, Sorisole, Italy) used a three-step program (5 min from room temperature to 180°C, 10 min hold at 180°C, 20 min cooling and ventilation). After cooling, the digested sample solutions were quantitatively transferred into 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 Qc, Thermo Scientific, Bremen, Germany), equipped with a collision cell and operating in the kinetic energy discrimination (KED) mode, was used to determine the ^{111}Cd isotope. A five-point calibration curve (including zero) was constructed for the quantitative analysis. Multielement internal standard (^{6}Li, ^{45}Sc, 10 ng mL^{-1}; ^{71}Ga, ^{89}Y and ^{209}Bi, 2 ng mL^{-1}) was introduced inline by an additional line through the peristaltic pump. Measured levels were corrected for the response factors of internal standards. The quality of the analytical process was verified by analysing of the certified reference material NIST SRM 1577c (Gaithersburg, MD, USA). Reference material was prepared in the same way as samples using microwave digestion. Replicate analyses were in the ranges of certified values.

Statistical analysis

Statistical analysis of experimental data was performed using Minitab® 17.1.0 Statistical Software. One-way analysis of variance (ANOVA) and Tukey's test were used for comparison of cadmium levels between muscle tissue and liver within different years.

Results and Discussion

Tables 2 and 3 show the measured cadmium levels in liver and leg muscle of common pheasant.

National legislation does not prescribe maximum levels (MLs) for cadmium in pheasant tissue. Therefore, we used MLs for cadmium in poultry tissue (liver, muscle) for compliance assessment; 0.50 mg kg^{-1} for liver and 0.050 mg kg^{-1} for muscle (*Official Gazette of RS, 2014*).

Table 2. Cadmium levels in liver of common pheasant from Serbia

Year	Number of samples	Mass fraction mg kg^{-1}			Non-compliant*
		Median	Range	Mean ± SD	
2018	89	0.203	0.025–4.206	0.354±0.511	17
2019	71	0.179	0.016–1.237	0.247±0.228	11
2020	92	0.226	0.005–1.324	0.306±0.274	18
2021	75	0.197	0.009–1.396	0.303±0.296	6.7

Legend:* Percentage of samples exceeding the permitted cadmium values defined for poultry by national legislation, which for liver is 0.5 mg kg^{-1}.

Table 3. Cadmium levels in leg muscle of common pheasant from Serbia

Year	Number of samples	Mass fraction mg kg^{-1}			Non-compliant*
		Median	Range	Mean±SD	
2018	89	0.005	< LOD–0.146	0.009±0.018	3.4
2019	71	0.006	0.006–0.036	0.006±0.007	/
2020	92	0.005	< LOD–0.235	0.011±0.026	/
2021	75	0.006	0.001–0.048	0.011±0.012	/

Legend: * Percentage of samples exceeding the permitted cadmium values defined for poultry by national legislation, which for muscle is 0.05 mg kg^{-1}.

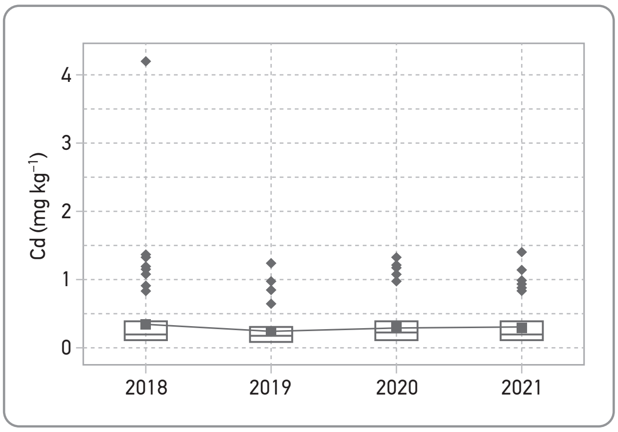

Figure 1. Cadmium levels (mg kg^{-1}) in pheasant liver samples

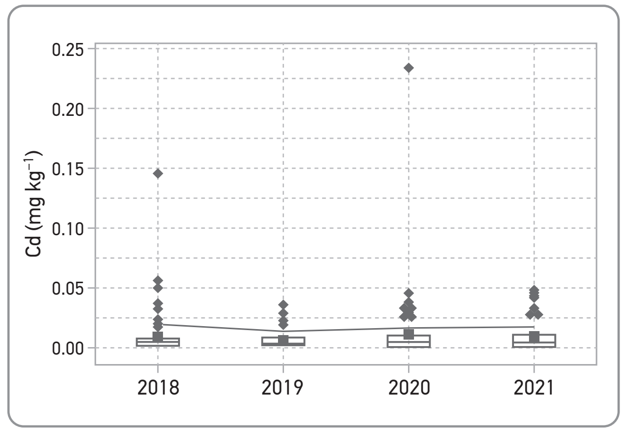

Figure 2. Cadmium levels (mg kg^{-1}) in pheasant leg muscle samples

For the entire observed period (2018–2021), levels of cadmium in liver were within the range of 0.005–4.206 mg kg^{-1}. The highest cadmium level in liver was determined in 2018 (4.206 mg kg^{-1}) (Figure 1). Comparing cadmium levels in liver for the four years, there was no statistically significant difference at a confidence level of 95%. The percentage of samples that were non-compliant with the ML for cadmium in liver was highest in 2020 (18.5%), and decreased in the following order: 2018>2019>2021 (Table 2).

The highest mean cadmium level in leg muscle was measured in 2020 (0.235 mg kg^{-1}) (Figure 2). Only 3.4% of all leg muscle samples exceeded the ML. At a confidence level of 95%, there was no statistically significant difference in leg muscle cadmium levels, comparing all four years. The limit of detection (LoD) for cadmium was 0.001 mg kg^{-1}.

As expected, comparing the results between liver and leg muscle, there was a statistically significant difference. *Nikolic et al.* (2017) reported a level of 0.262 mg kg^{-1}, but for the period from 2018 to 2021 (the current study), the mean cadmium level was 0.306 mg kg^{-1}. Therefore, the mean cadmium level in the liver of pheasants in Serbia was slightly higher in the period from 2018 to 2021 than in previous years (2013–2016). The mean cadmium level in the leg muscle of pheasants for the period 2018–2021 was 0.009 mg kg^{-1}, which was also higher than in 2013–2016 (0.006 mg kg^{-1}). *Dzugan et al.* (2012) reported a higher mean cadmium level in pheasant liver (Table 1) than was found in our study. The cadmium level in liver reported by *Celechovska et al.* (2008) (Czech Republic) was lower than the level in pheasant caught in Serbia.

Flis et al. (2020) reported that leg muscle samples from wild pheasant contained 0.012 mg kg^{-1} of cadmium, while farmed pheasant had 0.016 mg kg^{-1}, which means that the animals on the farms consumed feed with a higher cadmium content than in the food of wild birds. Both tested groups had higher cadmium levels in muscle than did birds in our findings in Serbia. Unlike our results, *Gasparik et al.* (2010) found 0.0336 mg kg^{-1} of cadmium in leg muscle of pheasants from Slovakia, which was a significantly higher level than our findings.

Conclusion

This study reports a slight increasing trend (numerical but not statistical) of cadmium content in the liver and muscles of pheasants, from 2014 onwards. Although such increase is not alarming, further research is recommended for the purposes of monitoring cadmium levels in the environment. Based on these findings, it is apparent that continuous biomonitoring of heavy metals is necessary in industrial areas since it is a useful indicator of the state of the environment. Firstly, this is because pheasant is used in human nutrition, and secondly, the data are useful to monitor concentrations of bioavailable cadmium that enters the food chain. Further research could be expanded to analysis of soil, air and water from pheasants' habitats.

Fazan kao sredstvo za biomonitoring nivoa kadmijuma u životnoj sredini u Srbiji

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A p s t r a k t: Hrana kontaminirana teškim metalima je problem koji je sve češći kod zemalja u razvoju. Jedan od teških metala označen kao zagađivač životne sredine je kadmijum. Ptice predstavljaju dobar izbor za biomonitoring, posebno kod područja gde je prisutan stacionarni izvor zagađenja. Uzorci fazana su prikupljeni tokom četiri lovne sezone u Srbiji u okviru programa Nacionalnog praćenja rezidua od 2018. do 2021. godine. Ukupan broj uzoraka je iznosio 327. Količina kadmijuma u uzorcima je određena primenom indukovano spregnute plazme sa masenim detektorom (eng. Inductively coupled plasma mass spectrometry — ICP-MS). Prosečna vrednost kadmijuma u analiziranim uzorcima jetre i mišića nogu je 0.306 mg kg^{-1} i 0.009 mg kg^{-1}. Opseg nivoa kadmijuma je bio 0.005–4.206 mg kg^{-1} za jetru i < 0,001–0.235 za mišić nogu. Nivo kadmijuma kod fazanu u Srbiji je neznatno povećan brojčano (ne statistički) u protekle četiri godine, pa nivo kadmijuma treba i dalje pažljivo pratiti.

***Ključne reči:** kadmijum, biomonitoring, fazan, ICP-MS.*

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References

Aloupi, M., Karagianni, A., Kazantzidis, S. & Akriotis, T. (2017). Heavy Metals in Liver and Brain of Waterfowl from the Evros Delta, Greece. *Archives of Environmental Contamination and Toxicology,* 72, 215–234.

Bernard, A. (2008). Cadmium & its adverse effect on human health. *Indian Journal of Medical Research,* 128, 557–564.

CABI (2015). Invasive Species Compendium. European Red List Status, *Phasianus colchicus* (common pheasant). (https://www.cabi.org/isc/datasheet/70470#tohabitat)

Celechovska O., Malota, L. & Zima, S. (2008). Entry of Heavy Metals into Food Chains a 20-year Comparison Study in Northern Moravia (Czech Republic). *Acta Veterinarica Brno,* 77, 645–652.

Dzugan, M., Zielinska, S., Heclik J., Pieniazek, M. & Szostek, M. (2012). Evaluation of heavy metals environmental contamination based on their concentrations in tissues of wild pheasant (*Phasianus colchicus* L.). *Journal of Microbiology, Biotechnology and Food Sciences,* 2 (1), 238–245.

EFSA, 2012. Cadmium dietary exposure in the European population. Scientific Report of EFSA. *EFSA Journal,* 10 (1), 2551, 1–37.

Flis, M., Grela, R., E., Zukowska, G. & Gugala, D. (2020). Nutritional composition and heavy metal content in breast and thigh muscles of wild and intensively reared common pheasants (*Phasianus colchicus*). *Journal of Veterinary Research,* 64, 305–312.

Florijancic, T., Opacak, A., Boskovic, I., Jelkić, D., Ozimec, S., Bogdanovic, T., Listes, I., Škrivanko, M. & Puskadija, Z. (2009). Heavy metal concentrations in the liver of two wild duck species: influence of species and gender. *Italian Journal of Animal Science,* 8 (3), 222–224.

Gasparik, J., Vladarova, D., Capcarova, M., Smehyl, P., Slamecka, J., Garaj, P., Stawarz, R. & Massanyi, P. (2010). Concentration of lead, cadmium, mercury and arsenic in leg skeletal muscles of three species of wild birds. *Journal of Environmental Science and Health Part A* 45, 818–823.

Govind, P. & Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Science,* 2 (2), 17–23.

Kekkonen, J., Hanski I., K., Vaisanen R., A. & Brommer, J. E. (2012). Levels of heavy metals in House Sparrows (*Passer domesticus*) from urban and rural habitats of southern Finland. *Ornis Fennica,* 89, 91–98.

Kim, J. & Oh, J. M., (2013). Assessment of trace metals in four bird species from Korea. *Environmental Monitoring and Assessment,* 185, 6847–6854.

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.

Mateo, R. & Guitart, R. (2003). Heavy Metals in Livers of Waterbirds from Spain. *Archives of Environmental Contamination and Toxicology,* 44, 398–404.

Medunic, G., Kuharic, Z., Fiket, Z., Bajramovic, M., Singh, A., L., Krivohlavek, A., Goran Kniewald, G. & Dujmovic, L. (2018). Selenium and other potentially toxic elements in vegetables and tissues of three non-migratory birds exposed to soil, water, and aquatic sediment contaminated with seleniferous Raša coal. *The Mining-Geology-Petroleum Engineering Bulletin,* 53–62.

Milaimi A., P., Selimi, Q., Letaj, K., Trebicka, A. & Milaimi, A. (2016). Accumulation of Heavy Metals in Feral Pigeons Living Near a Ferronickel Smelter. *Polish Journal of Environmental Studies,* 25 (6), 2695–2699.

Nikolic, D., Djinovic-Stojanovic, J., Stefanović S., Radičević, T., Trbović, D., Spirić, D., Janković. S. (2017). Distribution of cadmium in leg muscle and liver of game birds from Serbia. *IOP Conf. Series: Earth and Environmental Science* 85*,* 1–4.

Official Gazette of RS. (2014). No 29. National legislation of maximum concentrations of certain contaminants in food.

Swiergosz, R. & Kowalska, A. (2000). Cadmium accumulation and its effects in growing Pheasants *Phasianus colchicus* (L.). *Environmental Toxicology and Chemistry,* 19 (11), 2742–2750.

VKM (2015). Risk assessment of dietary cadmium exposure in the Norwegian population. Opinion of the Panel on Contaminants of the Norwegian Scientific Committee for Food Safety. *VKM Report* 2015, 12, Oslo, Norway.

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