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THE BROWN HARE (*LEPUS EUROPAEUS*) AS A BIOMONITOR FOR THE PRESENCE OF LEAD AND CADMIUM IN VOJVODINA, SERBIA



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SUMMARY

Lead (Pb) and cadmium (Cd) are heavy metals considered possible contaminants of water, soil and air (especially when occurring as a consequence of human activities in agriculture or industry), and can be found in the liver or kidneys of animals, thus serving as indicators of pollution. Vojvodina is a northern province of the Republic of Serbia which boasts very intensive agricultural production, especially crop production. The brown hare is well-adapted and abundant in Vojvodina. As a complete herbivore, it has the potential to be a good biomonitor for the presence of lead and cadmium in the chosen biotope. A total of 60 samples of hare livers were collected and analyzed immediately after hunting. The average concentration of lead in all the liver samples analyzed was 851.43 µg/kg of wet tissue. The lead concentrations determined at three different sites in Vojvodina (namely Odžaci in Bačka, Rusko selo in Banat and Kraljevci in Srem) were not statistically significant. However, the average lead concentrations at all the sites were above the permitted level (500 µg/kg of wet tissue). The maximum amount of lead determined was 2,302 µg/kg of wet tissue, i.e. almost five times above the permitted level. The cadmium concentrations in all the samples analyzed ranged from 12.90 to 1183.00 µg/kg of wet tissue. The average cadmium concentrations determined at three different sites were found statistically significant, suggesting a local source of contamination. The average cadmium concentrations at sites I, II and III were 65.57, 205.91 and 413.99 µg/kg of wet tissue, respectively. The average cadmium concentrations determined at the sites considered were below the permitted level, although exceeding the legal limit in some of the samples analyzed.

Key words:

brown hare, liver, heavy metals, lead, cadmium

INTRODUCTION

Lead (Pb) and cadmium (Cd) are heavy metals or pollutants found in nature as a consequence of natural or human activities. A number of natural events such as volcanic eruptions or meteorite falls can cause an increase in the concentration of lead and cadmium. However, human activities are most significantly and directly implicated in the elevated concentrations of lead and cadmium found in soil, water or air.

Vojvodina is a northern province of the Republic of Serbia which boasts very intensive agricultural production, especially crop production. Contemporary agriculture is plagued with the excessive use of chemicals in fertilizers, herbicides and pesticides. The soil, air and water near highways with heavy traffic, lead smelters, heavy industry or thermal power plants can have increased levels of heavy metals (Petkovšek et al., 2014; Spahić et al., 2018). Lead and cadmium from the soil can transfer to aquatic environments or be absorbed by plants. By this route, they enter the food chain and can be found as residues in animals. In modern and developed countries, pollution and transfer of pollutants such as heavy metals from soil, air or water have become a common concern.

Small mammals are very good biomonitors for the presence of heavy metals in nature. Biomonitors are used as indicators of the environment they inhabit. Free-range animals or wildlife depend on the water, soil and air available in their natural habitat. Moreover, herbivores are better biomonitors because their use for this purpose is not limited by the food chain effects (Tataruch & Kierdorf, 2003). The use of omnivores is limited due to the nature of their nutrition, which can cause cumulative effects.

The brown hare (*Lepus europaeus* Pall.) is widespread and abundant in Vojvodina, and, as a complete herbivore, it is considered an appropriate biomonitor for heavy metals, as suggested by previous studies (Tataruch & Kierdorf, 2003; Myslek & Kalisinska, 2006; Petkovšek et al., 2014; Lazarus et al., 2014; Beuković et al., 2015a; Beuković et al., 2019; Beuković et al., 2022). On account of this trait, the brown hare was selected for the purpose of this research. The brown hare is a game animal which is quite well-adapted to agricultural biotopes. Their home range size can vary considerably (from 21 to 330 ha), depending on the landscape type (Schai-Braun & Hackländer, 2014). For the purpose of this research, a total of three different sites were chosen: one in each of the three Vojvodinian regions (namely Banat, Bačka and Srem).

Lead and cadmium are heavy metals without any biological function in the animal body. During lifetime, both metals accumulate in animal tissues, especially the liver and kidneys. Once lead or cadmium are absorbed and deposited in bones, their half-life in humans is around 10-30 years (EFSA, 2009; EFSA, 2010). Cadmium is primarily toxic to the kidneys (EFSA, 2009). Animal organs such as the liver and kidneys are good indicators of the presence of heavy metals in the environment as there is a clear connection between cadmium concentrations in the environment and animal organs (Lindén et al., 2003; Reddy et al., 2017; Davidov et al., 2019).

MATERIAL AND METHODS

The brown hare liver sampling was carried out in the fall of the 2017 hunting season at three different sites in Vojvodina, Serbia: Odžaci (Bačka, N 45°31'36" E 19°15'42"), Rusko selo (Banat, N 45°45'29" E 20°34'37") and Kraljevci (Srem, N 44°54'5" E 19°51'5"), Fig. 1. After hunting, the livers of brown hares were sampled for analysis as a target tissue in which heavy metals accumulate during life. A total of 60 liver samples were collected: 20 samples at each site. Using a food blender, the samples were fragmented to a uniform pulp without large, unmodified pieces. The material was packed in clean and uncontaminated plastic zip bags, each of which was assigned a sample number for entering in the sample register. Prior to analysis, the samples were stored in deep-freeze conditions at -18 °C.



Figure 1. The brown hare liver sampling sites in Vojvodina (Serbia): 1. Kraljevci (Srem), 2. Rusko selo (Banat) and 3. Odžaci (Bačka)

Approximately a total of 1 g of the liver sample was weighed using an analytical balance and placed in a tube where it was digested by wet ashing with nitric and hydrochloric acid mixtures (3:1, v/v). The tube was subsequently heated

in a thermal block at 120 °C for 2 hours. The content of lead was determined by the electrothermal atomic absorption spectroscopy, using the method validated by Beuković et al. (2015b). In addition to the calculation of descriptive statistics parameters, the analysis of variance (ANOVA) and the post-hoc Tukey HSD test were performed.

RESULTS AND DISCUSSION

The average concentration of lead in all the samples analyzed was 851.43 µg/kg of wet weight (ww). The average lead concentrations at all three sites were similar without statistically significant differences, ranging from 727.2 to 977.46 µg/kg of wet tissue (Tab. 1). Both the minimum and maximum lead concentrations were determined in the brown hare liver samples collected at Site I: 288.00 µg/kg of wet tissue and 2,302.00 µg/kg of wet tissue, respectively.

Table 1. The lead concentrations in the brown hare livers sampled at three different sites in Vojvodina (µg/kg ww)

Site	I (Srem)	II (Banat)	III (Bačka)	Σ
Average	977.46 ^a	849.12 ^a	727.2 ^a	851.43
Minimum	288.00	449.80	257.10	257.10
Maximum	2,302.00	2,176.00	1,521.00	2,302.00
Median	957.65	808.70	673.60	741.80
Legend: ^{a, b} - statistically different (p < 0.05), Rows followed by the same letter do not differ significantly according to post-hoc Turkey HSD test				

The average cadmium concentration in all the brown hare liver samples collected at Sites I, II and III was 229.15 µg/kg (Tab. 2). The average cadmium concentrations in the brown hare livers sampled at Site I (Srem), II (Banat) and III (Bačka) were 65.57 µg/kg, 205.91 µg/kg and 413.99 µg/kg, respectively. Statistically significant differences were found in the concentration of lead in the brown hare liver samples depending on the collection site. The samples collected at Site III had the highest average cadmium concentration of 413.99 µg/kg. The maximum cadmium concentration of 1,183.00 µg/kg was determined in the sample collected at Site III.

Table 2. The cadmium concentrations in the brown hare livers sampled at three different sites in Vojvodina (µg/kg ww)

Site	I (Srem)	II (Banat)	III (Bačka)	Σ
Average	65.57 ^a	205.91 ^a	413.99 ^b	229.15
Minimum	12.90	40.18	48.14	12.90
Maximum	145.20	863.70	1,183.00	1,183.00
Median	68.95	136.45	312.45	119.30
Legend: ^{a, b} - statistically different (p < 0.05), Rows followed by the same letter do not differ significantly according to post-hoc Turkey HSD test				

The mean and minimum lead levels obtained were similar to the results of Beuković et al. (2019), who analyzed the samples collected in the southern part of Vojvodina (Srem). The mean level of lead determined was 788.75 µg/kg, the minimum level of lead was 259 µg/kg, and the maximum level of lead was 2,821 µg/kg.

The average lead level in the brown hare liver samples obtained in this study (851.43 µg/kg) was similar to that reported by Beuković et al. (2022), i.e. 838 µg/kg. The maximum level (ML) of lead permitted in the edible tissue of animals in Serbia is 500 µg/kg (RS Pravilnik, 2011). The average lead concentration determined in all the samples analyzed exceeded the ML fourfold. However, free living game animals tend to have increased levels of lead in their edible tissues and muscles (Petrović, 2007; Bilandžić et al., 2010; Lazarus et al., 2014). Moreover, free-range domestic animals (e.g. the Mangulica breed) usually have higher concentrations of some heavy metals such as lead and cadmium in their tissues than fattening pigs reared under controlled conditions (Nikolić et al., 2017). This can be accounted for by the fact that free-range animals have access to a variety of food and water sources, thus having more opportunities to come in contact with the sources of pollution. However, when compared to some other studies in the EU countries, the average lead value obtained for all the samples in our study was much higher. In a study in Slovakia, the mean lead value determined in the hare liver sample was 221 µg/kg, whereas the maximum lead value was 1,196 µg/kg (Massanyi et al., 2003). Moreover, in one previous study of the presence of heavy metals in the liver of pigs in Vojvodina, the lead concentrations obtained exceeded the ML in 3 out of 20 liver samples analyzed (Polovinski-Horvatović et al., 2021). It seems that the presence of lead as a result of pollution in Vojvodina requires further research for prospective solutions.

Upon absorption, lead and cadmium deposit primarily in the hare's liver and kidneys. The concentration of cadmium in the kidneys is several times higher than that in the liver (Jokanović et al., 2013; Tomović et al., 2015). We found

that the average concentration of cadmium in the samples collected at all three experimental sites was 229.15 µg/kg, which is similar to the average concentration of cadmium reported in our previous study, i.e. 243 µg/kg (Beuković et al., 2022). Statistically significant differences were found between the average cadmium concentrations in the hare liver samples collected at Sites I, II and III. The concentration of cadmium in wild boar livers sampled in Slovakia ranged from 190-1920 µg/kg of wet weight, with a median of 474 µg/kg of wet weight (Gašparík et al., 2017). The average cadmium concentration determined in a total of 150 hare liver samples, collected at nine different sites in Vojvodina, was 231.1 µg/kg, with a maximum concentration of 900.9 µg/kg (Beuković et al. 2015a). The range of cadmium values obtained herein is slightly higher, most probably due to the sites selected. However, the average concentration of cadmium in all the samples analyzed is below the permitted level of 500 µg/kg (RS Pravilnik, 2011). It is observable that cadmium levels were lower than lead levels. However, cadmium values were found to exceed the permitted level in some samples, suggesting a possible local source of contamination.

CONCLUSION

The results obtained in this study indicate that the lead and cadmium concentrations determined in the brown hare livers sampled at three different sites in Vojvodina were above the quantification limit. The lead levels determined were higher than cadmium levels and mostly exceeded the ML, which is a cause for concern. There were no statistically significant differences between the lead concentrations in the samples collected in the three regions of Vojvodina, indicating increased levels of lead in hare livers across the province. However, the presence of cadmium in the samples analyzed depended on the site, indicating a local source of pollution. The concentrations of these two heavy metals in hare livers are indicative of pollution. Vojvodina is the hub of agricultural activity in the Republic of Serbia. Future research is needed to determine the source of lead and cadmium pollution in the agricultural biotopes of Vojvodina.

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Conflict of interest: The authors declare that they have no conflict of interest.

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