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Heavy Metals (As, Cd, Hg and Pb) in Hare Tissues: A Survey

Teški metali (As, Cd, Hg i Pb) u tkivima zeca: pregled

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HEAVY METALS (As, Cd, Hg AND Pb) IN HARE TISSUES: A SURVEY

Gulin, J.⁽¹⁾, Florijančić, T.⁽²⁾, Bilandžić, N.⁽³⁾, Ozimec, S.⁽²⁾, Bošković, I.⁽²⁾, Lončarić, Z.⁽²⁾

Scientific review

Pregledni znanstveni članak

SUMMARY

Concerns have been escalating over the increase in heavy metal levels in the environment due to anthropogenic impacts. Toxic heavy metals (As, Cd, Hg and Pb) are especially dangerous, as they negatively affect organisms and cause outbreaks of diseases. The hare has been proven to be a good indicator of environmental heavy metal contamination. The liver and kidney are the tissues most commonly used in biomonitoring. Hares inhabiting a contaminated habitat have higher concentrations of heavy metals in these tissues than those from a referential habitat. As is mostly accumulated in the nails and hair, Cd in the kidney, Hg in the brain and kidney, and Pb in the brain and diaphragm. Cd and Hg concentrations in hare liver and kidney increased with animal age. In most countries, hare meat is safe for human consumption, while the consumption of entrails is not recommended.

Keywords: bioaccumulation, environmental pollution, hare, heavy metals, *Lepus europaeus*, tissues

INTRODUCTION

The present-day consumerism lifestyle and tendency to maximize profits exert a negative impact on the environment. Metals are naturally present in the environment, are not degraded but instead circulate in nature in various oxidational and chemical forms (Fairbrother et al., 2007). Anthropogenic processes (urbanization, industrialization, transportation, and agriculture) effectuate an increase in heavy metal concentrations in the environment. The main sources of heavy metals in the atmosphere are metal production and processing, fossil fuel combustion that releases large quantities of certain metals, and the production and use of metal products (Nriagu, 1990). Mining areas are more contaminated areas compared to other areas in industrial regions while food crop plantations areas are more contaminated areas in agricultural regions (Yang et al., 2018; Wu et al., 2022). The usage of mineral fertilizers and pesticides directly increases the heavy metal concentrations in soils (Atafar et al., 2010; Tariq et al., 2016; Qin et al., 2021). To reduce contamination there are different remediation technologies (Vareda et al., 2019; Radočaj et al., 2020). Due to an adverse effect on human, animal, and plant health, the concern over increasing heavy metal concentrations is escalating worldwide (Jaishankar et al., 2014). Once they enter the environment, heavy metals are not degraded but are accumulated in the superficial soil strata and in the

water (Nriagu, 1990). Plants uptake heavy metals via the roots (Efroymsen et al., 2004) or via atmospheric deposition onto the foliage. Animals and human's intake heavy metals by respiration, directly via skin, and via nutrition, which is the most significant source of heavy metal intake into the body (Fairbrother et al., 2007). Heavy metals are transferred from a lower to a higher level of the food chain and are bioaccumulated in certain tissues (Kaplan et al., 2011; Govind and Madhuri, 2014).

The toxicity of a specific heavy metal is differentiated depending on the dosage and duration of exposure, species, age, sex, and the environmental and nutritional factors of an individual. The absorption, distribution, transformation, and excretion of heavy metals in the organism depend on its chemical form, and the capacity of an organism to excrete and/or accumulate the metal (Fairbrother et al., 2007; Tchounwou et al., 2012). The negative effects of heavy metals on an organism are usually not easily recognizable, as most exposure is to low dosages over the long term. Studies have established that chronic exposure to heavy metals causes mutagenicity, carcinogenicity, teratogenicity, nephrotoxicity,

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neurotoxicity, immunosuppression, poor overall condition and a reduced reproduction capacity (Valko et al., 2005; Tchounwou et al., 2012; Govind and Madhuri, 2014). Arsenic, cadmium, mercury and lead have no known biological function, are toxic at low concentrations, and are thus classified among the most dangerous elements on Earth (ATSDR, 2017).

To determine the threat posed by pollutants to human health, biomonitoring is implemented with the aim of serving as an early warning system against environmental disturbances. Wild animals feeding on natural feed are particularly useful in biomonitoring, as they provide the best information about habitat contamination (Wren, 1986; Tataruch and Kierdorf, 2003). To correctly interpret and compare results, knowledge is needed on the species diet specificities, lifestyle, sampling period (seasonal differences) and age of individuals. The liver and kidney are the most frequently used organs for biomonitoring (Tataruch and Kierdorf, 2003). The hare has been proven to be a good bioindicator of a habitat contamination by heavy metals in Europe.

The objective of this study is to present a literature review of the use of hare as a bioindicator of toxic metals (As, Cd, Hg, and Pb), to compare the results of these studies, to explain the bioaccumulation specificities of these metals in hare tissues, and to assess the risk of human consumptions of hare meat and entrails.

HARE AS A BIOINDICATOR OF ENVIRONMENTAL POLLUTION

After entering the organism, a part of the heavy metals is excreted in the feces, by urine, or through skin in sweat and part is accumulated in the tissues. The liver and kidney, as the organs important for organism detoxification, accumulate high levels of heavy metals (Nordberg, 1998). Some metals (Cd, Cu, Hg, Ni and Zn) accumulate in the kidney during an animal's lifespan, while an accumulation in the liver represents a metal intake in a shorter time span (months), as detected by Scheuhammer (1987). Wajdzik et al. (2017) state that the hare liver is the best indicator of heavy metal contamination in comparison to all other tissues.

Many studies have confirmed higher heavy metal concentrations in hare tissues from contaminated habitats than in reference habitats. Kálás et al. (1995) examined heavy metal concentrations in the liver and/or kidneys of different animal species (including the mountain hare, *Lepus timidus*) along the Norwegian border in the vicinity of a Russian industrial facility for nickel production and smelting. The highest levels of Cu, Ni, and Cr were found in hare tissues adjacent to the facility. Amuno et al. (2016) found that arctic hare (*Lepus arcticus*) inhabiting the vicinity of a former lead (Pb) and zinc (Zn) mine had increased concentrations of Pb and Cd in the liver as opposed to those arctic hares in an unpolluted reference habitat and that the concentration of soil-borne heavy metals decreased with increasing distance from the contamination source. In the hare tissues from Koprivnica-Križevci County, Croatia, where natural gas is produced and agriculture is intensive (with the usage of mineral fertilizers), hare tissues contained higher levels

of Cd and Hg than on the island of Krk, where agriculture and industry are not developed (Linšak et al., 2013; Tomić Linšak et al., 2014). Wajdzik et al. (2017) found the higher levels of Cd and Pb in the hare livers in an industrial area in relation to the hares from an agricultural and forested area. Petrović (2013) concluded that the accumulation of heavy metals in hare tissues is site-specific or regionally specific, and dependent on the source of heavy metal emissions. Beuković et al. (2018) concluded that the level of lead and cadmium in hare liver depending on the type of anthropogenic effects. According to this conclusion exploitation of ore is the largest pollutant of the environment while intensive agriculture has the least impact on the occurrence of elevated levels of cadmium and lead in hare liver in relation to other anthropogenic effects. Durkalec et al. (2015) analyzed levels of toxic metal (Cd, Hg, and Pb) in tissues of wild boar and roe deer. Their results indicate that the area affected by metal smelting was more contaminated than brown coal mining area and the reference site, as indicated by higher levels of Pb and Cd in tissues and stomach contents of the animals.

The literature states that metals may be naturally occurring in the soil (Pedersen and Lierhagen, 2006; Wajdzik, 2006), and may also be deposited on plants from the atmosphere (Kálás et al., 2000). Soil acidity favors the accumulation of certain metals in the plants (Lončarić et al., 2008).

The differences in the heavy metal accumulation levels in hares may also be the consequence of differing dietary patterns. Coprophagy contributes to an increase in heavy metal accumulation in hare tissues (Komiš and Ballová, 2021). Venäläinen et al. (1996) established higher levels of examined metals in the tissues of the mountain hare than in the tissues of the European hare (*Lepus europaeus*). The mountain hare draws nourishment more from the ligneous (perennial) plants that contain higher concentrations of heavy metals than the European hare, which mostly feeds on annual plants. According to Fidalgo et al. (2016), Iberian hares (*Lepus granatensis*) inhabiting the cereal field areas had lower heavy metal concentrations than hares inhabiting areas with bushy and perennial vegetation.

Higher metal levels in hare tissues have been found in the hibernial period (Massányi et al., 2003; Škrivanko et al., 2008). Some authors emphasize the higher heavy metal concentrations in female individuals, as females are fed more, for they are bred several times a year (Ahmed et al., 2016).

Wajdzik (2006) did not detect differences in metal concentrations in the blood of hares from the territories with differing contamination levels. Kolesarova et al. (2008) detected heavy metal concentrations in the liver and kidney but did not detect any correlations with biochemical and hormonal parameters in blood plasma. Bukovjan et al. (1997) detected an increased percentage of pathohistological alterations in certain organs (brain, kidney, liver, and spleen) of hares from an industrial area in comparison to hares from uncontaminated habitats. Hares from a contaminated habitat are easier prey for predators and are less productive (Halecki et al., 2017). Chronic exposure to heavy metals causes oxidative stress in animals which may act negatively on a health condition and provoke the onset of a disease (Tomić Linšak et al., 2014).

Many factors affect the metal levels in wildlife tissues. The results of metal levels in the same tissues, among animals of the same species, of the same age groups that inhabit a similar biotope, are comparable. By comparing metal levels in tissues of free-living animals (red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), European hare, wild boar (*Sus scrofa*), Eurasian badger (*Meles meles*), brown bear (*Ursus arctos*), red fox (*Vulpes vulpes*), and grey wolf (*Canis lupus*)) living in the same area, the authors (Berzas Nevado et al., 2012; Bakowska et al., 2016; Malová et al., 2019; Nawrocka et al., 2020; Pilarczyk et al., 2020; Cebulska et al., 2021) claim that the most burdened animal species was wild boar. Bilandžić et al. (2012) observed the differences in metal exposure (As, Cd, Cu, Pb, and Hg) in the muscle, liver and kidney tissues of brown bears, grey wolves, Eurasian lynx (*Lynx lynx*), Eurasian badgers and pine martens (*Martes martes*) from Croatia. The results presented that the Eurasian badger accumulated the highest levels of elements. Variability in animal species contamination is a consequence of their distinct way of life, food intake and food composition. Wild boar and badger, as typical omnivores obtaining food mainly through digging of soil looking for roots, tubers, and invertebrates are more exposed to environmental pollution than herbivores and carnivores (Pilarczyk et al., 2020). Malová et al. (2019) analyzed metal levels in the samples of biotope components and established that contamination was in the following descending order: moss – bark – grass – leaf litter – needles.

ARSENIC (As)

Arsenic has a high affinity to sulfhydryl groups and is primarily accumulated in the nails (Amuno et al., 2017) and hair (Bukovjan et al., 2016) of hares as tissues rich in keratin. Bukovjan et al. (2016) established that the ratio of arsenic concentration in the muscle and other tissues and excrement was 1:2.96 for liver, 1:4.35 for kidney, 1:1.07 for heart, 1:2.73 for lungs, 1:3.3 for testicles, 1:5.90 for bones, 1:114.68 for hair and 1:60.05 for feces, with no differences determined between the sexes. Amuno et al. (2017) examined the As and Cd concentrations in hare tissues from the vicinity of a gold mine (Yellowknife, Canada) and a reference site. Soilborne As concentrations fluctuated between 35.2 and 1636.2 mg/kg and from 1.3 to 59.4 mg/kg in the willow specimens. At a distance from 14 to 22 km from the mine, these levels fluctuated from 10.1 to 75.3 mg/kg in the soil and from 1.1 to 3 mg/kg in the willow specimens. As concentrations were 17.8 to 48.9 times higher in the stomach contents and 4 to 23 times higher in the nails from the mine territory than in the same hare tissues from the reference site. Strong correlations were found between As levels in nails, liver, and stomach contents, and these findings were higher than other reports from Europe and Canada (Table 1). In Croatia, no significant As concentrations in hare tissues were recorded (Škrivanko et al., 2008).

Table 1. As concentrations (mg/kg of wet weight) in tissues of hare species by location

Tablica 1. Koncentracije As (mg/kg mokre mase) u tkivima različitih vrsta zeca prema lokaciji

Species / Vrsta	Location / Lokacija	Samples / Uzorci	Liver / Jetra	Kidney / Bubrež	Muscle / Mišić	Reference / Referenca
			x (min-max)	x (min-max)	x (min-max)	
<i>L. americanus</i>	Labrador, Canada (2014)	p (n=10)	0.0105 (bdl-0.015)	0.0105 (bdl-0.015)	bdl	Intrinsik, (2015)
<i>L. americanus</i>	Yellowknife, Canada	p (n=10)A	0.76 (0.19–2.30)	1.11 (0.334–4.0)	-	Amuno et al. (2017)
		u (n=10)A	0.2 (0.015–0.766)	0.28 (0.023–0.945)	-	
<i>L. arcticus</i>	Nunavut, Canada	p (n=8)	bdl-4.79	bdl (n=3)	-	Amuno et al. (2016)
		u (n=3)	bdl-7.32	bdl-3.78 (n=2)	-	
<i>L. europaeus</i>	Tynec, Czech Republic (1988 – 1995)	p (n=15) A	0.12 (0.017-0.58)	0.091 (0.009-0.22)	0.023 (0.005-0.084)	Bukovjan et al. (1997)
<i>L. europaeus</i>	Czech Republic (2009 – 2013)	u (n=105) A	0.01893 (0.0046-0.097)	0.02636 (0.00198-0.184)	0.0082 (0.0024-0.060)	Bukovjan et al. (2016)
<i>L. europaeus</i>	Eastern Croatia	winter (n=41)	-	bdl	bdl	Škrivanko et al. (2008)
		spring (n=30)	-	0.025	bdl	
<i>L. europaeus</i>	Lviv region, Ukraine	(n=8)	0.044 (0.032-0.064)	0.055 (0.031-0.080)	0.051 (0.030-0.057)	Pilarczyk et al. (2020)

p – polluted area, u – unpolluted area, A – adult hares, bdl – below detection limit / p - zagađeno, u – nezagađeno, A – adultni zečevi, bdl – ispod limita detekcije

CADMIUM (Cd)

In the tissues, Cd is bound to metallothionein and transferred further to the kidney (Massányi et al., 2003.). The kidney is a target tissue for Cd accumulation and excretion from the organism. Such results were established in hares (Kramárová et al., 2005a; Kolesarova et al., 2008; Shahid et al., 2013) and also in other domesticated (Toman and Massányi, 1996; Kramárová

et al., 2005b) and wild animals (Falandysz et al., 2005; Srebočan et al., 2011; Gašparik et al., 2017), and in humans (Yoo et al., 2002). The significant differences between Cd levels in the liver and kidney between adult and juvenile hares (Massányi et al., 2003; Pedersen and Lierhagen, 2006; Shahid et al., 2013; Fidalgo et al., 2016) is the consequence of the biologic half-life of Cd of more than ten years, with Cd levels increasing with age (Petrović et al., 2014).

Table 2. Cd concentrations (mg/kg of wet weight) in tissues of hare species by location

Tablica 2. Koncentracije Cd (mg/kg mokre mase) u tkivima različitih vrsta zeca prema lokaciji

Species / Vrsta	Location / Lokacija	Samples / Uzorci	Liver / Jetra	Kidney / Bubrež	Muscle / Mišić	Reference / Referenca
			x (min-max)	x (min-max)	x (min-max)	
<i>L. americanus</i>	Labrador, Canada (2014)	p (n=10)	0.174 (0.0366-0.562)	4.55 (0.448-11.7)	0.00335 (ild-0.0056)	Intrinsik, (2015)
<i>L. americanus</i>	Yellowknife, Canada	p (n=10) A	0.49 (0.096-1.092)	10.8 (2.544-22.28)	-	Amuno et al. (2017)
		u (n=10) A	0.2 (0.09-0.49)	2.98 (1.76-6.41)	-	
<i>L. arcticus</i>	Nanavut, Canada (2003) *	u (n=7) J	0.196 (0.021-0.440)	1.73 (0.29-4.41)	0.005 (0.002-0.009)	Pedersen and Lierhagen (2006)
		u (n=9) A	4.58 (1.68-10.90)	106.6 (55.2-219.9)	0.082 (0.035-0.201)	
<i>L. arcticus</i>	Nunavut, Canada	p (n=8)	(0.018-3.44)	-	-	Amuno et al. (2016)
		u (n=3)	(0.087-1.94)	-	-	
<i>L. capensis</i>	Pakistan *	u (n=21) A	1.5 (0.2-3.2)	1.53 (0.2-3.5)	1.27 (0.1-3.4)	Ahmed et al. (2016)
<i>L. europaeus</i>	Tynec, Czech Republic (1988-95)	p (n=15) A	1.07 (0.28-2.38)	1.954 (0.38-4.61)	0.155 (0.04-0.31)	Bukovjan et al. (1997)
<i>L. europaeus</i>	Finland (1980-82)	p (n=88)	0.330	3,83	0.008 (n=38)	Venäläinen et al. (1996)
		u (n=15)	0.171	1.46 (n=13)	0.003 (n=2)	
	Finland (1992-93)	p (n=28)	0.160	1,91	0.003 (n=26)	
		u (n=3)	0.057	0.627	0.001	
<i>L. europaeus</i>	Eastern Croatia	winter (n=41)	-	0.938 (bdl-9.588)	bdl	Škrivanko et al. (2008)
		spring (n=30)	-	0.707 (bdl-6.139)	bdl	
<i>L. europaeus</i>	Molve/Krk, Croatia **	p (n=5) A	1.054 (0.449-2.453)	16.594 (3.396-40.040)	0.037 (0.023-0.259)	Tomčić Linšak et al. (2014)
		u (n=5) A	0.763 (0.514-0.904)	3.090 (2.952-5.058)	0.033 (0.017-0.047)	
<i>L. europaeus</i>	Malopolska, Poland (1996 – 2001)	p + u (n=164) J+A	1.65 (0.13-6.98)	14.7 (0.96-83.36)	-	Wajdzik (2006)
<i>L. europaeus</i>	Czluchow, Poland (1988 – 2001)	u (n=64) 25j+39A	0.55	5,63	0.01	Myslek and Kalisinska (2006)
<i>L. europaeus</i>	Malopolska, Poland	1 (2006) (n=10) A	1.99 (1.14-2.85)	17.83 (4.10-37.98)	0.12 (0.06-0.23)	Halecki et al. (2017)
		2 -II- (n=10)A	1.12 (0.16-2.05)	7.91 (1.50-15.10)	0.08 (0.04-0.28)	
		3 -II- (n=10)A	1.30 (0.31-2.61)	10.09 (2.32-18.58)	0.07 (0.04-0.13)	
		1 (2007) (n=10) A	1.86 (1.16-3.19)	22.39 (6.28-49.12)	0.10 (0.06-0.16)	
		2 -II- (n=10)A	1.27 (0.28-2.21)	9.41 (2.21-19.00)	0.07 (0.04-0.16)	
		3 -II- (n=10)A	1.21 (0.08-2.75)	9.56 (2.17-17.61)	0.07 (0.04-0.13)	
<i>L. europaeus</i>	Malopolska, Poland *	p + u (n=35) J+A	5,7	61,7	-	Wajdzik et al. (2017)
<i>L. europaeus</i>	Nitra, Slovakia	u (n=5) A	0.36	2,05	-	Toman and Massányi (1996)
<i>L. europaeus</i>	Trnava, Slovakia ***	(n=74) 26J+48A	0.160 (0.003-1.004)	1.570 (0.004-4.719)	-	Massányi et al. (2003)
<i>L. europaeus</i>	Serbia (2010 – 11)	(n=156) 69J+87A	0.17 (0.01-0.85)	1.83 (0.06-7.54)	-	Petrović (2013)
<i>L. europaeus</i>	Vojvodina, Serbia (2017)	(n=122) J	0.208 (0-1.414)	-	-	Beuković et al. (2022)
		(n=74) A	0.303 (0.02-1.254)	-	-	
<i>L. europaeus</i>	Kirikale, Turkey (2013 – 14)	p (n=15) A	0.83	4,49	1,19	Demirbas and Enduran (2017)
<i>L. europaeus</i>	Lviv region, Ukraine	(n=8)	0.089 (0.053-0.191)	0.719 (0.088-2.08)	0.049 (0.042-0.066)	Pilarczyk et al. (2020)
<i>L. granatensis</i>	Spain	(n=36) J	0.06 (0.01-0.27)	0.34 (0.02-1.80)	-	Fidalgo et al. (2016)
		(n=29) A	0.09 (0.01-0.26)	0.69 (0.04-2.16)	-	
<i>L. nigricollis</i>	Pakistan	(n=31) 28A+3J	6.09 (2.55-9.90)	7.33 (2.50-17.10)	2.70 (0.85-4.75)	Shahid et al. (2013)
<i>L. timidus</i>	Finland (1980 – 82)	p (n=65)	0.485	11,1	0.014 (n=36)	Venäläinen et al. (1996)
		u (n=36)	0.390	4.55 (n=35)	0.010 (n=7)	
	Finland (1992 – 93)	p (n=43)	0.445	10,7	0.006 (n=39)	
		u (n=8)	0.185	3.73 (n=7)	0.005	
<i>L. timidus</i>	Norway *, **	p (n=14) J	0.53	3,9	-	Kälås et al. (1995)
		p (n=4) A	1,7	33	-	
		u (n=3) J	0.43	1,9	-	
		u (n=2) A	0.61	16	-	

p – polluted area, u – unpolluted area, A – adult hares, J – juvenile hares, bdl – below detection limit, * – concentrations in mg/kg of dry weight, ** – median (min. – max.), l – polluted location, 2 and 3 – less polluted location, *** – specimens collected throughout the year /

p – zagađeno, u – nezagađeno, A – adultni zečevi, J – juvenilni zečevi, bdl – ispod limita detekcije, * – koncentracije u mg/kg suhe mase, ** – medijan (min-max), 1 – zagađena lokacija, 2 i 3 – manje zagađena lokacija, *** – uzorci prikupljeni kroz cijelu godinu

Cd accumulation in hare tissues according to age groups was examined by Petrović et al. (2013). They found significant differences in Cd levels in the kidney and liver between all age groups. The Cd concentration ratio in the kidney in relation to the liver increases with age in all age groups, and Cd concentrations in the kidney were positively correlated with concentrations in the liver (Petrović et al., 2013; Ahmed et al., 2016).

Amuno et al. (2016) established a strong correlation between soilborne Cd and Cd levels in hare kidney. High soilborne Cd levels may be caused by local geology (Pedersen and Lierhagen, 2006), and also by intensive agriculture (Tomić Linšak et al., 2014). Wajdzik (2006) confirmed Cd bioaccumulation, with levels in the liver and kidney several times higher than those in the soil and plants. Some authors report higher Cd concentrations in tissues of female than in males' tissues (Massányi et al., 2003; Škrivanko et al., 2008; Shahid et al., 2013).

The highest Cd concentrations in hare kidney were recorded in Canada (Pedersen and Lierhagen, 2006) and in Poland's Lesser Poland (Małopolska) province (Wajdzik,

2006; Halecki et al., 2017; Wajdzik et al., 2017). Similar Cd concentrations were established in hares in Finland (Venäläinen et al., 1996), Czech Republic (Bukovjan et al., 1997), Slovakia (Toman and Massányi, 1996; Massányi et al., 2003), and Serbia (Petrović et al., 2013). The lowest concentrations were recorded in the tissues of the Iberian hare in Spain (Fidalgo et al., 2016). In Croatia, the highest median values were found in the hare kidney from the Molve area (16.544 mg/kg), followed by the hares from the island of Krk (3.090 mg/kg), while the lowest values were found in hares from eastern Croatia (0.938 mg/kg), as described by Škrivanko et al. in 2008 (Table 2).

MERCURY (Hg)

Of the total quantity consumed, usually less than 10% of Hg is absorbed. The Hg half-life time in organs fluctuates from days to months, and it is mostly retained in the brain and the kidneys (Linšak et al., 2013). The intake of soilborne Hg is very limited, with plant Hg concentrations significantly lower than in the soil, while atmospheric deposition is the main source of Hg for plants (Millhollen et al., 2006).

Table 3. Hg concentrations (mg/kg of wet weight) in tissues of hare species by location

Tablica 3. Koncentracije Hg (mg/kg mokre mase) u tkivima različitih vrsta zeca prema lokaciji

Species / Vrsta	Location / Lokacija	Samples / Uzorci	Liver / Jetra	Kidney / Bubreg	Muscle / Mišić	Reference / Referenca
			x (min-max)	x (min-max)	x (min-max)	
<i>L. americanus</i>	Labrador, Canada (2014)	p (n=10)	0.0129 (0.0025-0.0362)	0.116 (0.0426-0.369)	0.00228 (bdl-0.005)	Intrinsic, (2015)
<i>L. arcticus</i>	Nanavut, Canada (2003) *	u (n=7) J	0.016 (0.007-0.034)	0.201 (0.087-0.488)	-	Pedersen and Lierhagen (2006)
		u (n=9) A	0.172 (0.060-0.349)	0.541 (0.123-1.066)	0.002 (0.002-0.003)	
<i>L. europaeus</i>	Tynec, Czech Republic (1988-1995)	p (n=15)A	0.132 (0.009-0.406)	0.278 (0.039-0.993)	0.02 (0.005-0.059)	Bukovjan et al. (1997)
<i>L. europaeus</i>	Eastern Croatia	winter (n=41)	-	0.030 (bdl-0.326)	-	Škrivanko et al. (2008)
		spring (n=30)	-	0.011 (bdl-0.057)	-	
<i>L. europaeus</i>	Molve, Croatia **	p (n=15)A (1984-90)	0.074	0.179	0.002	Špirić et al. (2012)
		p (n=10)A (1990-08)	0.018	0.050	0.001	
<i>L. europaeus</i>	Molve, Croatia	p (n=5)A	(0.058-0.189)	(0.138-0.406)	(0.013-0.046)	Linšak et al. (2013)
<i>L. europaeus</i>	Trnava, Slovačka ***	(n=74) 26J+48A	0.021 (0-0.193)	0.030 (0-0.332)	-	Massányi et al. (2003)
<i>L. europaeus</i>	Serbia (2010-11)	(n=156)69J+87A	0.02 (0.006-0.068)	0.046 (0.006-0.261)	-	Petrović (2013)
<i>L. europaeus</i>	Kirikkale, Turkey (2013-14)	p (n=15)A	0.06	0.10	-	Demirbas and Enduran (2017)
<i>L. timidus</i>	Norway *, **	p (n=14) J	0.025	0.170	-	Kålås et al. (1995)
		p (n=4) A	0.055	0.359	-	
		u (n=3) J	0.010	0.065	-	
		u (n=2) A	0.024	0.145	-	

p – polluted area, u – unpolluted area, A – adult hares, J – juvenile hares, bdl – below detection limit, * – concentrations in mg/kg of dry weight, ** – median (min-max.), 1 – polluted location, 2 and 3 – less polluted location, *** – specimens collected throughout the year

p – zagađeno, u – nezagađeno, A – adultni zečevi, J – juvenilni zečevi, bdl – ispod limita detekcije, * – koncentracije u mg/kg suhe mase, ** – medijan (min-max), 1 – zagađena lokacija, 2 i 3 – manje zagađena lokacija, *** – uzorci prikupljeni kroz cijelu godinu

Higher Hg concentrations were established in the kidney than in the liver (Bukovjan et al., 1997; Špirić et al., 2012; Petrović, 2012; Demirbaş and Erduran, 2017) and in adult hares compared with juvenile individuals (Kålås et al., 1995; Pedersen and Lierhagen, 2006). Berzas Nevado et al. (2012) found similar results in the tissues of wild boar and red deer, and Srebočan et al. (2011) in

the tissues of roe deer and wild boar. Berzas Nevado et al. (2012) found a significant correlation between Hg concentrations in the tissues of wild boar and red deer and distance to Hg mining district. Petrović et al. (2014) reported that bioaccumulation of Hg in the hare liver is visible throughout the hare's lifecycle.

Study areas in Croatia (Škrivanko et al., 2008; Špirić et al., 2012) have low Hg contamination levels and are comparable with study areas in Slovakia (Massányi et al., 2003) and Serbia (Petrović, 2013). The concentrations in hare tissues in the Czech Republic (Bukovjan et al., 1997) were several times higher. In the Molve area of Croatia, Hg levels in the liver and kidney decreased between two sampling periods (1984–90 vs. 1990–2008; cf. Špirić et al., 2012), which was attributed to the cessation of the

use of Hg fungicides and the installation of a purification system in the nearby natural gas production and processing facility.

LEAD (Pb)

The highest Pb concentrations were measured in the brain (10.9 mg/kg), followed by the diaphragm, liver, and hare kidney (Wajdzik et al., 2017).

Table 4. Pb concentrations (mg/kg of wet weight) in the tissues of hare species by location

Tablica 4. Koncentracije Pb (mg/kg mokre mase) u tkivima različitih vrsta zeca prema lokaciji

Species / Vrsta	Location / Lokacija	Samples / Uzorci	Liver / Jetra	Kidney / Bubrež	Muscle / Mišić	Reference / Referenca
			x (min-max)	x (min-max)	x (min-max)	
<i>L. americanus</i>	Labrador, Canada (2014)	p (n=10)	0.0104 (0.0191-0.281)	0.0598 (0.0189-0.114)	0.00239 (bdl-0.0035)	Intrinsik, (2015)
<i>L. arcticus</i>	Nunavut, Canada (2003) *	u (n=7) J	0.233 (0.046–0.468)	0.447 (0.137–1.247)	0.011 (0.005–0.019)	Pedersen and Lierhagen (2006)
		u (n=9) A	0.200 (0.009–0.363)	0.223 (0.122–0.413)	0.011 (0.005–0.019)	
<i>L. arcticus</i>	Nunavut, Canada	p (n=8)	(0.08-1.15)	-	-	Amuno et al. (2016)
		u (n=3)	(0.1-0.16)	-	-	
<i>L. capensis</i>	Pakistan *	u (n=21)A	28.67 (1.00-116.4)	19.68 (6.30-28.20)	25.61 (0.30-139.0)	Ahmed et al. (2016)
<i>L. europaeus</i>	Tynec, Czech Republic (1988-1995)	p (n=15)	0.399 (0.05-0.69)	0.413 (0.05-0.73)	0.115 (0.05-0.2)	Bukovjan et al. (1997)
<i>L. europaeus</i>	Finland (1980-82)	p (n=88)	1.,01	0.90	0.13 (n=31)	Venäläinen et al. (1996)
		u (n=15)	0.47 (n=14)	0.76	0.08 (n=2)	
	Finland (1992-93)	p (n=28)	0.17	0.19	0.05 (n=26)	
		u (n=3)	0.06	0.05	0.01	
<i>L. europaeus</i>	Malopolska, Poland (1996-01)	p + u (n=164) J+A	1.24 (0.30-3.63)	1.20 (0.22-6.30)	-	Wajdzik (2006)
<i>L. europaeus</i>	Czuchow, Poland (1998-2001)	u (n=64) 25J+39A	1,15	0.83	0.28	Myslek and Kalisinska (2006)
<i>L. europaeus</i>	Malopolska, Poland	1 (2006) (n=10)A	1.83 (0.87-3.53)	1.47 (0.26-2.24)	0.27 (0.19-0.38)	Halecki et al. (2017)
		2 -II- (n=10)A	0.89 (0.33-1.78)	0.85 (0.22-1.90)	0.11 (0.04-0.21)	
		3 -II- (n=10)A	1.11 (0.64-1.92)	0.89 (0.54-1.28)	0.19 (0.08-0.32)	
		1 (2007) (n=10)A	1.55 (0.49-2.10)	1.63 (0.84-2.86)	0.27 (0.19-0.36)	
		2 -II- (n=10)A	0.84 (0.43-1.43)	0.87 (0.22-1.76)	0.09 (0.04-0.13)	
		3 -II- (n=10)A	0.83 (0.35-1.41)	1.22 (0.54-3.22)	0.17 (0.04-0.38)	
<i>L. europaeus</i>	Tmava, Slovakia ***	(n=74) 26J+48A	0.221 (0.013-1.196)	0.115 (0.013-0.721)	-	Massányi et al. (2003)
<i>L. europaeus</i>	Serbia (2010-11)	(n=156) 69J+87A	0.22(0.06-1.72)	0.21 (0.06-1.12)	-	Petrović (2013)
<i>L. europaeus</i>	Vojvodina, Serbia (2017)	(n=122) J	0.838 (0.06-3.248)	-	-	Beuković et al. (2022)
		(n=74) A	0.841 (0.11-3.70)	-	-	
<i>L. europaeus</i>	Kirikkale, Turkey (2013-14)	p (n=15) A	2,19	1,23	7,83	Demirbas and Enduran (2017)
<i>L. europaeus</i>	Lviv region, Ukraine	(n=8)	0.674 (0.625-0.780)	0.680 (0.620-0.765)	0.691 (0.645-0.751)	Pilarczyk et al. (2020)
<i>L. granatensis</i>	Spain	(n=36) J	0.10 (0.03-0.26)	0.09 (0.002-0.23)	-	Fidalgo et al. (2016)
		(n=29) A	0.08 (0.03-0.23)	0.07 (0.01-0.17)	-	
<i>L. nigricollis</i>	Pakistan	(n=31) 28A+3J	9.53 (3.53-19.37)	8.34 (0.37-16.49)	3.99 (1.12-7.22)	Shahid et al. (2013)
<i>L. timidus</i>	Finland (1980-82)	p (n=65)	0.59	0.91	0.13 (n=28)	Venäläinen et al. (1996)
		u (n=35)	0.59	0.71	0.05 (n=7)	
	Finland (1992-93)	p (n=43)	0.29	0.63	0.07 (n=32)	
		u (n=8)	0.23	0.21 (n=7)	0.04 (n=4)	
<i>L. timidus</i>	Norway *, **	p (n=14) J	0.52	0.67	-	Kålås et al. (1995)
		p (n=4) A	0.67	0.40	-	
		u (n=3) J	0.15	0.15	-	
		u (n=2) A	1,64	0.52	-	
<i>L. timidus</i>	Norway *	(n=49) J	1.15 (0.15-4.20)	-	-	Kålås et al. (2000)
		(n=71) A	0.55 (0.15-7.07)	-	-	

p – polluted area, u – unpolluted area, A – adult hares, J – juvenile hares, bdl – below detection limit, * – concentrations in mg/kg of dry weight, ** – median (min. – max.), 1 – polluted location, 2 and 3 – less polluted location, *** – specimens collected throughout the year

p – zagađeno, u – nezagađeno, A – adultni zečevi, J – juvenilni zečevi, bdl – ispod limita detekcije, * – koncentracije u mg/kg suhe mase, ** – medijan (min-max), 1 – zagađena lokacija, 2 i 3 – manje zagađena lokacija, *** – uzorci prikupljeni kroz cijelu godinu

Median Pb concentration values in the liver and kidney were similar, with slightly higher concentrations recorded in the liver (Massányi et al., 2003; Shahid et al., 2013; Halecki et al., 2017). No increases in Pb concentrations in liver or kidney with age were determined (Wajdzik, 2006; Petrović, 2013; Shahid et al., 2013), though some studies reported higher concentrations in the liver of juvenile individuals than in adults (Massányi et al., 2003; Wajdzik, 2006; Srebočan et al., 2011), and in males than in females (Massányi et al., 2003; Shahid et al., 2013).

The main source of Pb intake for herbivores is the Pb transfer to plants by atmospheric deposition, and significant correlations between soilborne Pb levels and those in hare tissues were reported in territories without deposition contamination (Kálás et al., 2000). The root system prevents more significant Pb transfer to the aerial plant parts, and Wajdzik (2006) established that Pb levels in hare tissues were several times lower than in soil.

Pb concentrations in hare liver and kidney was significantly decreased in Finland over a period of a decade, which was attributed to the usage of fuels with a lower Pb content (Venäläinen et al., 1996). A high Pb level (40.31 mg/kg) in the lungs of Pakistani hares indicates the presence of air pollution by lead (Ahmed et al., 2016), and high environmental pollution (Shahid et al., 2013, Table 4).

Pb concentrations in the hare tissues in Canada are low due to the low values of atmospheric Pb deposition (Pedersen and Lierhagen, 2006). In the European hare, the lowest concentrations were recorded in Finland (Venäläinen et al., 1996), Slovakia (Massányi et al., 2003), and Serbia (Petrović, 2013), slightly higher concentrations in the Czech Republic (Bukovjan et al., 1997), and the highest concentrations in Poland (Wajdzik, 2006; Myslek and Kalisinska, 2006). The results of Pb level in the hare tissues from Vojvodina, Serbia (Beuković et al., 2022), and the Lviv region, Ukraine (Pilarczyk et al., 2020) are cause for concern. In a study in Croatia, all values were below the detection limit (Škrivanko et al., 2008). The lowest concentrations of Pb, and Cd, were recorded in the Iberian hare (Fidalgo et al., 2016).

CONSUMPTION OF HARE MEAT AND ENTRAILS

Pursuant to the European standards, the maximum permitted Pb concentration in meat and entrails is 0.10 and 0.50 mg/kg of wet weight, respectively, and for Cd in the meat, liver, and kidney is 0.050, 0.50, and 1.0 mg/kg of wet weight, respectively (European Commission Regulation, 2006). There are no set levels for As and Hg concentrations in hare tissues. When research results

are cited in dry weight concentrations, it is necessary to convert them into the wet weights. Myslek and Kalisinska (2006) reported that the mean water content of hare kidney, liver, and muscle was 78.1, 77.1, and 75.7%, retrospectively. Škrivanko et al. (2008) stated that hare muscle contains on average 75.45% water.

The values measured in the meat and entrails (liver and kidney) of hares in Pakistan (Shahid et al., 2013; Ahmed et al., 2016) and Turkey (Demirbaş and Erduran, 2017) exceeded these stipulated limits and therefore are not suitable for human consumption. The permissible concentration of Pb was exceeded in all meat and entrails samples of roe deer, wild boar, and hare in western Ukraine (Pilarczyk et al., 2020). The average concentration of Pb in the hare liver was in the permitted level only in 2 out of 17 locations in Vojvodina, Serbia (Beuković et al., 2022).

In certain meat specimens from the Czech Republic (Bukovjan et al., 1997) and Poland (Myslek and Kalisinska, 2006; Halecki et al., 2017), the Cd and Pb values above the recommended limit were recorded. In all studies, values above the maximal limits were found in most liver and/or kidney specimens, and therefore, hare entrails are not recommended for human consumption. Because of the high percentage of free-living game (fallow deer, roe deer, red deer, wild boar, and brown bear) liver and kidney samples exceeding the legislative limits for cadmium and lead people should avoid the consumption of certain game species' entrails (Lazarus et al., 2014; Malmsten et al., 2021).

Škrivanko et al. (2008) reported that the values of heavy metals above the detection limit were not recorded in hare meat. Pb was not detected in the kidney in that study, while Cd concentrations were detected in 15 of 71 kidney samples. Tomić Linšak et al. (2014) found Cd concentrations higher than the recommended limits in both liver and kidney.

CONCLUSIONS

The hare is a good bioindicator of environmental contamination by heavy metals. The concentrations detected in the liver and kidney provide the best results concerning local pollution levels. Higher heavy metal concentrations have been recorded in hare tissues in the vicinity of a contamination source (industry, transportation) and in intensive agriculture areas. Hare meat is safe for human consumption, except in the countries with high contamination levels (Pakistan, Turkey, Poland, and the Czech Republic). Based on the literature data, the consumption of the entrails is not recommended.

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TEŠKI METALI (As, Cd, Hg I Pb) U TKIVIMA ZECA: PREGLED

SAŽETAK

Raste zabrinutost uslijed povećavanja razina teških metala u okolišu zbog antropogenoga utjecaja. Posebno su opasni toksični teški metali (As, Cd, Hg i Pb), koji negativno utječu na organizme i izazivaju pojavu bolesti. Zec se pokazao dobrim indikatorom zagađenja staništa teškim metalima. Za provedbu biomonitoringa najčešće se koriste jetra i bubreg. Zečevi koji obitavaju na zagađenome staništu imaju više koncentracije teških metala u tim tkivima negoli oni s referentnoga staništa. As se akumulira najviše u noktima i kosi, Cd u bubregu, Hg u mozgu i bubrezima, a Pb u mozgu i dijafragmi. Starenjem jedinke povećavaju se koncentracije Cd i Hg u jetri i bubregu zeca. Meso zeca je u većini zemalja sigurno za ljudsku prehranu, dok se korištenje iznutrica ne preporučuje.

Ključne riječi: bioakumulacija, zagađenje okoliša, zec, teški metali, *Lepus europaeus*, tkiva

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