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

Gulin, Josip; Florijančić, Tihomir; Bilandžić, Nina; Ozimec, Siniša; Bošković, Ivica; Lončarić, Zdenko

Source / Izvornik: **Poljoprivreda, 2023, 29, 86 - 96**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.18047/poljo.29.2.11

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

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

Gulin, J., Florijančić, T., Bilandžić, N., Ozimec, S., Bošković, I., Lončarić, Z.

Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

https://doi.org/10.18047/poljo.29.2.11

Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

ISSN 1330-7142
UDK=637.55:546.95
https://doi.org/10.18047/poljo.29.2.11

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 (Efroymson 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,

(1) Josip Gulin, M Eng. Agr. – Public Institution of Krka National Park, Trg Ivana Pavla II. br. 5, 22000 Šibenik, Croatia, (2) Prof. Dr. Tihomir Florijančić, (tflorijanc@fazos.hr), Prof. Dr. Siniša Ozimec, Assoc. Prof. Dr. Ivica Bošković, Prof. Dr. Zdenko Lončarić – Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Vladimira Preloga 1, 31000 Osijek, Croatia, (3) Dr. sc. Nina Bilandžić – Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia

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. Kålås 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 (Kålås 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 (Kompiš 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 hibernal 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; Malòvá 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 wolfs, 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). Malòvá 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 / *Bubreg*	Muscle / *Mišić*	Reference / *Referenca*
			x (min-max)	x (min-max)	x (min-max)	
L. americanus	Labrador, Canada (2014)	p (n=10)	0.0105 (bdl-0.015)	0.0105 (bdl-0.015)	bdl	Intrinsik, (2015)
L. americanus	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)	-	
L. arcticus	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)	-	
L. europaeus	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)
L. europaeus	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)
L. europaeus	Eastern Croatia	winter (n=41)	-	bdl	bdl	Škrivanko et al. (2008)
		spring (n=30)	-	0.025	bdl	
L. europaeus	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; Gašparík 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 / *Bubreg*	Muscle / *Mišić*	Reference / *Referenca*
			x (min-max)	x (min-max)	x (min-max)	
L. americanus	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)
L. americanus	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)	-	
L. arcticus	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)	
L. arcticus	Nunavut, Canada	p (n=8)	(0.018-3.44)	-	-	Amuno et al. (2016)
		u (n=3)	(0.087-1.94)	-	-	
L. capensis	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)
L. europaeus	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)
L. europaeus	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	
L. europaeus	Eastern Croatia	winter (n=41)	-	0.938 (bdl-9.588)	bdl	Škrivanko et al. (2008)
		spring (n=30)	-	0.707 (bdl-6.139)	bdl	
L. europaeus	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)	Tomić 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)	
L. europaeus	Malopolska, Poland (1996 – 2001)	p + u (n=164) J+A	1.65 (0.13-6.98)	14.7 (0.96-83.36)	-	Wajdzik (2006)
L. europaeus	Czluchow, Poland (1988 – 2001)	u (n=64) 25j+39A	0.55	5,63	0.01	Myslek and Kalisinska (2006)
L. europaeus	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)	
L. europaeus	Malopolska, Poland *	p + u (n=35) J+A	5,7	61.7	-	Wajdzik et al. (2017)
L.europaeus	Nitra, Slovakia	u (n=5) A	0.36	2,05	-	Toman and Massányi (1996)
L. europaeus	Trnava, Slovakia ***	(n=74) 26J+48A	0.160 (0.003-1.004)	1.570 (0.004-4.719)	-	Massányi et al. (2003)
L. europaeus	Serbia (2010 – 11)	(n=156) 69J+87A	0.17 (0.01-0.85)	1.83 (0.06-7.54)	-	Petrović (2013)
L. europaeus	Vojvodina, Serbia (2017)	(n=122) J	0.208 (0-1.414)	-	-	Beuković et al. (2022)
		(n=74) A	0.303 (0.02-1.254)	-	-	
L. europaeus	Kirikkale,Turkey (2013 – 14)	p (n=15) A	0.83	4,49	1,19	Demirbas and Enduran (2017)
L. europaeus	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)
L. granatensis	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)	-	
L. nigricollis	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)
L. timidus	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	
L. timidus	Norway *, **	p (n=14) J	0.53	3,9	-	Kålås 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)	
L. americanus	Labrador, Canada (2014)	p (n=10)	0.0129 (0.0025-0.0362)	0.116 (0.0426-0.369)	0.00228 (bdl-0.005)	Intrinsik, (2015)
L. arcticus	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)	
L. europaeus	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)
L. europaeus	Eastern Croatia	winter (n=41)	-	0.030 (bdl-0.326)	-	Škrivanko et al. (2008)
		spring (n=30)	-	0.011 (bdl-0.057)	-	
L. europaeus	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	
L. europaeus	Molve, Croatia	p (n=5)A	(0.058-0.189)	(0.138-0.406)	(0.013-0.046)	Linšak et al. (2013)
L. europaeus	Trnava, Slovačka ***	(n=74) 26J+48A	0.021 (0-0.193)	0.030 (0-0.332)	-	Massányi et al. (2003)
L. europaeus	Serbia (2010-11)	(n=156)69J+87A	0.02 (0.006-0.068)	0.046 (0.006-0.261)	-	Petrović (2013)
L. europaeus	Kirikkale, Turkey (2013-14)	p (n=15)A	0.06	0.10	-	Demirbas and Enduran (2017)
L. timidus	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.), I – 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 / *Bubreg*	Muscle / *Mišić*	Reference / *Referenca*
			x (min-max)	x (min-max)	x (min-max)	
L. americanus	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)
L. arcticus	Nanavut, 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)	
L. arcticus	Nunavut, Canada	p (n=8)	(0.08-1.15)	-	-	Amuno et al. (2016)
		u (n=3)	(0.1-0.16)	-	-	
L. capensis	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)
L. europaeus	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)
L. europaeus	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	
L. europaeus	Malopolska, Poland (1996-01)	p + u (n=164) J+A	1.24 (0.30-3.63)	1.20 (0.22-6.30)	-	Wajdzik (2006)
L. europaeus	Czluchow, Poland (1998-2001)	u (n=64) 25J+39A	1,15	0.83	0.28	Myslek and Kalisinska (2006)
L. europaeus	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 (022-1.90)	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)	
L. europaeus	Trnava, Slovakia ***	(n=74) 26J+48A	0.221 (0.013-1.196)	0.115 (0.013-0.721)	-	Massányi et al. (2003)
L. europaeus	Serbia (2010-11)	(n=156) 69J+87A	0.22(0.06-1.72)	0.21 (0.06-1.12)	-	Petrović (2013)
L. europaeus	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)	-	-	
L. europaeus	Kirikkale, Turkey (2013-14)	p (n=15) A	2,19	1,23	7,83	Demirbas and Enduran (2017)
L. europaeus	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)
L. granatensis	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)	-	
L. nigricollis	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)
L. timidus	Finland (1980-82)	p (n=65)	0.59	0.91	0.13 (n=28)	Venäläinenet 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)	
L. timidus	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	-	
L. timidus	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.), 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

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 (Kålås 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; Mysłek and Kalisinska, 2006). The results of Pb level in the hare tissues from Vojvodina, Serbia (Beuković et al., 2022), and the Lviv region, Ukraine (Pilarzcyk 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. Mysłek 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 (Mysłek 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.

REFERENCES

1. Agency for Toxic Substances and Disease Registry (ATSDR). (2017). *The ATSDR 2017 Substance Priority List*. Retrieved from https://www.atsdr.cdc.gov/spl/
2. Ahmed, M.S., Azam, M.A., Ahmed, K.S. & Ali, H. (2016). Accumulation of Some Heavy Metals in Selected Tissues of Cape Hare, *Lepus capensis* from Pakistan. *Pakistan Journal of Wildlife, 7*(2), 11-20.
3. Amuno, S., Niyogi, S., Amuno, M. & Attitaq, J. (2016). Heavy Metal Bioaccumulation and Histopathological Alterations in Wild Arctic Hares (*Lepus arcticus*) Inhabiting a Former Lead-Zinc Mine in the Canadian High Arctic: A Preliminary Study. *Science of the Total Environment, 556*, 252–263. https://doi.org/10.1016/j.scitotenv.2016.03.007
4. Amuno, S., Jamwal, A., Grahn, B. & Niyogi, S. (2017). Chronic Arsenicosis and Cadmium Exposure in Wild Snowshoe Hares (*Lepus americanus*) Breeding Near Yellowknife, Northwest Territories (Canada), Part 1: Evaluation of Oxidative Stress, Antioxidant Activities and Hepatic Damage. *Science of the Total Environment, 618*, 916-926. https://doi.org/10.1016/j.scitotenv.2017.08.278
5. Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, M. & Mahvi, H. (2010). Effect of Fertilizer Application on Soil Heavy Metal Concentration. *Environmental Monitoring and Assessment, 160*, 83–89. https://doi.org/10.1007/s10661-008-0659-x
6. Bąkowska, M., Pilarczyk, B., Tomza-Marciniak, A., Udała, J. & Pilarczyk, R. (2016) The bioaccumulation of lead in the organs of roe deer (*Capreolus capreolus* L.), red deer (*Cervus elaphus* L.), and wild boar (*Sus scrofa* L.) from Poland. *Environmental Science and Pollution Research, 23*(14), 14373-14382. doi: 10.1007/s11356-016-6605-5.
7. Berzas Nevado, J.J., Rodríguez Martín-Doimeadios, R.C., Mateo, R., Rodríguez Farinas, N., Rodríguez-Estival, J., & Patino Ropero, M.J. (2012) Mercury exposure and mechanism of response in large game using the Almaden mercury mining area (Spain) as a case study. *Environmental Reserch, 112*, 58-66. https://doi.org/10.1016/ j.envres.2011.09.019.
8. Beuković, D., Popović, Z. & Beuković, M. (2018) Exposure of brown hare (*Lepus europaeus* P.) population to harmful effect of lead (Pb) and cadmium (Cd) in the food chain due to anthropogenic factors. Conference: International Symposium on Animal Science (ISAS), Belgrade-Zemun, Serbia, 64–68.
9. Beuković, D., Vukadinović, M., Krstović, S., Polovinski-Horvatović, M., Jajić, I., Popović, Z., Lavadinović, V. & Beuković, M. (2022) The European Hare (*Lepus europaeus*) as a Biomonitor of Lead (Pb) and Cadmium (Cd) Occurrence in the Agro Biotope of Vojvodina, Serbia. *Animals, 12,* 1249. https://doi.org/10.3390/ ani12101249
10. Bilandžić, N., Dežđek, D., Sedak, M., Đokić, M., Šimić, B., Rudan, N., Brstilo, M. & Lisicin, T. (2012) Trace elements in tissues of wild carnivores and omnivores in Croatia. *Bulletin of Environmental Contamination and Toxicology, 88*(1), 94–99. https://doi.org/10.1007/s00128-011-0449-y.
11. Bukovjan, K., Wittlingerová, Z. & Černá, E. (1997). Chemical Elements in Tissues and Histological Changes in Tissues of Hares (*L. europaeus* Pall.). *Scientia Agriculturae Bohemica, 28*, 215–226.
12. Bukovjan, K., Wittlingerová, Z. & Kutlvašr, K. (2016). Arsenic Deposition in Tissues of the European Hare (*Lepus europaeus*). *Acta Veterinaria Brno, 85*, 215–221. https://doi.org/10.2754/avb201685030215
13. Cebulska, K., Sobiech, P., Tobolski, D., Wysocka, D., Janiszewski, P., Zalewski, D., Gugołek, A. & Illek, J. (2021) Comparison of the content of selected heavy metals in the liver tissue of the wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*) and red deer (*Cervus elaphus*), living in north-eastern Poland. *Polish Journal of Veterinary Sciences, 24*(3), 425-432. doi: 10.24425/pjvs.2021.138734.
14. Demirbaş, Y. & Erduran, N. (2017). Concentration of Selected Heavy Metals in Brown Hare (*Lepus europaeus*) and Wild Boar (*Sus scrofa*) from Central Turkey. *Balkan Journal of Wildlife Research, 4*(2), 26–33. https://doi.org/10.15679/bjwr.v4i2.54
15. Durkalec, M., Kolacz, R., Opalinski, S., Nawrocka, A. & Zmudzki, J. (2015) Bioaccumulation of Lead, Cadmium and Mercury in Roe Deer and Wild Boars from Areas with Different Levels of Toxic Metal Pollution. *International Journal of Environmental Reserch 9*(1), 205–212. https://doi.org/10.22059/IJER.2015.890
16. Efroymson, R.A., Sample, B.E. & Suter, G.W. (2004). Bioaccumulation of Inorganic Chemicals from Soil by Plants: Spiked Soils vs. Field Contamination or Background. *Human and Ecological Risk Assessment, 10*(6), 1117–1127. https://doi.org/10.1080/10807030490887177
17. European Commission Regulation № 1881/2006. (2006). Setting Maximum Levels for Certain Contaminants in Foodstuff. *Official Journal of the European Union, 364*, 5–24. Retrieved from https://eur-lex.europa.eu/legal-content/HR/TXT/PDF/?uri=CELEX:32006R1881&from=EN
18. Fairbrother, A., Wenstel, R., Sappington, K. & Wood, W. (2007). Framework for Metals Risk Assessment. *Ecotoxicology and Environmental Safety, 68*(2), 145–227. https://doi.org/10.1016/j.ecoenv.2007.03.015
19. Falandysz, J., Szymczyk-Kobrzyńska, K., Brzostowski, A., Zalewski, K., & Zasadowski, A. (2005). Concentrations of Heavy Metals in the Tissues of Red Deer (*Cervus elaphus*) from the Region of Warmia and Mazury, Poland. *Food Additives and Contaminants, 22*(2), 141–49. https://doi.org/10.1080/02652030500047273
20. Fidalgo, L.E., de la Cruz, B., Goicoa, A. & Espino, L. (2016). Accumulation of Zinc, Copper, Cadmium and Lead in Liver and Kidney of the Iberian Hare (*Lepus granatensis*) from Spain. *Research & Reviews: Journal of Veterinary Sciences, 2*(1), 15–20.
21. Gašparík, J., Binkowski, L.J., Jahnátek, A., Šmehýl, P., Dobiaš, M., Lukáč, N., Błaszczyk, M., Semla, M. & Massányi, P. (2017). Levels of Metals in Kidney, Liver, and Muscle Tissue and Their Influence on the Fitness for the Consumption of Wild Boar from Western Slovakia. *Biological Trace Element Research, 177*(2), 258–266. https://doi.org/10.1007/s12011-016-0884-z
22. Govind, P. & Madhuri, S. (2014). Heavy Metals Causing Toxicity in Animals and Fishes. *Research Journal of Animal, Veterinary and Fishery Sciences, 2*(2), 17–23.
23. Halecki, W., Gąsiorek, M., Wajdzik, M., Pająk, M. & Kulak, D. (2017). Population Parameters Including

Breeding Season of the European Brown Hare (*Lepus europaeus*) Exposed to Cadmium and Lead Pollution. *Fresenius Environmental Bulletin, 26*(4), 2998–3004.

24. Intrinsik. (2015). *Baseline Country Food Study in the Vicinity of the Wabush Mine. Final Report*. Retrieved from https://www.mae.gov.nl.ca/env_assessment/projects/Y2013/1711/1711_app9_wabush3.pdf.
25. Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. & Beeregowda, K.N. (2014). Toxicity, Mechanism, and Health Effects of Some Heavy Metals. *Interdisciplinary Toxicology, 7*(2), 60–72. https://doi.org/10.2478/intox-2014-0009
26. Kålås, J.A., Ringsby, T.H. & Lierhagen, S. (1995). Metals and Selenium in Wild Animals from Norwegian Areas Close to Russian Nickel Smelters. *Environmental Monitoring and Assessment, 36*(3), 251-270. https://doi.org/10.1007/BF00547905
27. Kålås, J.A., Steinnes, E. & Lierhagen, S. (2000). Lead Exposure of Small Herbivorous Vertebrates from Atmospheric Pollution. *Environmental Pollution, 107*, 21–29. https://doi.org/10.1016/S0269-7491(99)00155-4
28. Kalpan, O., Yildirim, N.C., Yildirim, N. & Cimen, M. (2011). Toxic Elements in Animal Products and Environmental Health. *Asian Journal of Animal and Veterinary Advances, 6*(3), 228–232. https://doi.org/10.3923/ajava.2011.228.232
29. Kolesarova, A., Slamečka, J., Jurčík, R., Tataruch, F., Lukáč, N., Kováčik, J., Capcarová, M., Valent, M. & Massányi, P. (2008). Environmental Levels of Cadmium, Lead, and Mercury in Brown Hares and Their Relation to Blood Metabolic Parameters. *Journal of Environmental Science and Health A, 43*(6), 653–657. https://doi.org/10.1080/10934520801893741
30. Kompiš, M. & Ballová, Z.K. (2021). The influence of preferred habitat and daily range of the European hare on its contamination by heavy metals: a case study from the West Carpathians. *Environmental Science and Pollution Research 21*, 52093–52105. https://doi.org/10.1007/s11356-021-14363-x
31. Kramárová, M., Massányi, P., Slamečka, J., Tataruch, F., Jančová, A., Gašparík, J., … & Jurčík, R. (2005a). Distribution of Cadmium and Lead in Liver and Kidneys of Some Wild Animals in Slovakia. *Journal of Environmental Science and Health*, *40*(3), 593–600. https://doi.org/10.1081/ESE-200046605
32. Kramárová, M., Massányi, P., Jančová, A., Toman, R., Slamečka, J., Tataruch, F., … & Haščík, P. (2005b). Concentration of Cadmium in the Liver and Kidneys of Some Wild and Farm Animals. *Bulletin of the Veterinary Institute in Pulawy, 49*(4), 465–469.
33. Lazarus, M., Prevendar Crnić, A., Bilandžić, N., Kusak, J. & Reljić, S. (2014) Cadmium, lead, and mercury exposure assessment among Croatian consumers of free-living game. *Arhiv za higijenu rada i toksikologiju, 65* (3), 281-291. https://doi.org/10.2478/10004-1254-65-2014-2527
34. Linšak, Ž., Tomić Linšak, D., Špirić, Z., Srebočan, E., Glad, M. & Milin, Č. (2013). Effects of Mercury on Glutathione and Glutathione-Dependent Enzymes in Hares (*Lepus europaeus* Pallas). *Journal of Environmental Science and Health A, 48*(11), 325–1332. https://doi.org/10.1080/10934529.2013.781869
35. Lončarić, Z., Karalić, K., Popović, B., Rastija, D. & Vukobratović, M. (2008). Total and Plant-Available Micronutrients in Acidic and Calcareous Soils in Croatia. *Cereal Research Communications, 36*, 331–334.
36. Malmsten, A., Dalin, AM., Pettersson, J. & Persson, S. (2021) Concentrations of cadmium, lead, arsenic, and some essential metals in wild boar from Sweden. *European Journal of Wildlife Research, 67*,18. https://doi.org/10.1007/s10344-021-01460-y
37. Malòvá, J., Ciberej, J., Pavel Mala, P., Zigo, F. & Semjon, B. (2019) Heavy metals levels int he tissues of wild living animals from two distrinct industrially exsploited areas in Slovakia. *Slovak Journal of Animal Science*, *52* (3), 100–110.
38. Massányi, P., Tataruch, F., Slamečka, J., Toman, R. & Jurík, R. (2003). Accumulation of Lead, Cadmium, and Mercury in Liver and Kidney of the Brown Hare (*Lepus europaeus*) in Relation to the Season, Age, and Sex in the West Slovakian Lowland. *Journal of Environmental Science and Health A, 38*(7), 1299–1309. https://doi.org/10.1081/ese-120021127
39. Millhollen, A.G., Obrist, D. & Gustin, M.S. (2006). Mercury Accumulation in Grass and Forbs Species as a Function of Atmospheric Carbon Dioxide Concentrations and Mercury Exposures in Air and Soil. *Chemosphere, 65*(5), 889–897. https://doi.org/10.1016/j.chemosphere.2006.03.008
40. Mysłek, P. & Kalisinska, E. (2006). Contents of Selected Heavy Metals in the Liver, Kidneys, and Abdominal Muscle of the Brown Hare (*Lepus europaeus* Pallas) in Central Pomerania, Poland. *Polish Journal of Veterinary Sciences, 9*(1), 31–41.
41. Nawrocka, A., Durkalec, M., Szkoda, J., Filipek, A., Kmiecik, M., Zmudzki, J., & Posyniak, A. (2020) Total mercury levels in the muscle and liver of livestock and game animals in Poland, 2009–2018. *Chemosphere,* 258:127311. https://doi.org/10.1016/j.chemosphere. 2020.127311
42. Nordberg, M. (1998). Metallothioneins: Historical Review and State of Knowledge. *Talanta 46*(2), 243–254. https://doi.org/10.1016/S0039-9140(97)00345-7
43. Nriagu, J.O. (1990). Global Metal Pollution: Poisoning the Biosphere? *Environment 32*(7), 7–33. https://doi.org/10.1080/00139157.1990.9929037
44. Pedersen, S. & Lierhagen, S. (2006). Heavy Metal Accumulation in Arctic Hares (*Lepus arcticus*) in Nunavut, Canada. *Science of the Total Environment, 368*(2-3), 951–955. https://doi.org/10.1016/j.scitotenv.2006.05.014.
45. Petrović, Z. (2013). *Procena biomonitorskog potencijala zeca (Lepus europaeus) na osnovu akumulacije teških metala u tkivima*. Dissertation. University of Belgrade, Faculty of Veterinary Medicine, Beograd. Retrieved from https://nardus.mpn.gov.rs/handle/123456789/2987
46. Petrović, Z., Teodorović, V., Dimitrijević, M., Borozan, S., Beuković, M., Nikolić, D., & Spirić, D. (2013). Environmental Cd and Zn Concentrations in Liver and Kidney of European Hare from Different Serbian Regions. *Hemijska Industrija, 67*(4), 593–599. https://doi.org/10.2298/HEMIND120815100P
47. Petrović, Z., Teodorović, V., Djurić, S., Miličević, D., Vranić, D. & Lukić, M. (2014). Cadmium and Mercury

Accumulation in European Hare (*Lepus europaeus*): Age-Dependent Relationships in Renal and Hepatic tissue. *Environmental Science and Pollution Research, 21*, 14058–14068. https://doi.org/10.1007/s11356-014-3290-0

48. Pilarczyk, B., Tomza-Marciniak, A., Pilarczyk, R., Udała, J., Kruzhel, B. & Ligocki, M. (2020) Content of essential and non-essential elements in wild animals from western Ukraine and the health risks associated with meat and liver consumption. *Chemosphere, 244,* 125506. https://doi.org/10.1016/j.chemosphere.2019.125506.

49. Qin, G., Niu, Z., Yu, J., Li, Z., Ma, J. & Xiang, P. (2021) Soil heavy metal pollution and food safety in China: Effects, sources and removing technology. *Chemosphere, 267,*129205, 1-12. https://doi.org/10.1016/j.chemosphere.2020.129205.

50. Radočaj, D., Velić, N., Jurišić, M. & Merdić, E. (2020). The remediation of agricultural land contaminated by heavy metals. *Poljoprivreda 26(2),* 30-42. https://doi.org/10.18047/poljo.26.2.4

51. Scheuhammer, A.M. (1987). The Chronic Toxicity of Aluminium, Cadmium, Mercury, and Lead in Birds: A Review. *Environmental Pollution, 46*(4), 263–295. https://doi.org/10.1016/0269-7491(87)90173-4

52. Shahid, N., Anwar, S., Qadir, A., Ali, H., Suchentrunk, F. & Arshad, H.M. (2013). Accumulation of Some Selected Heavy Metals in *Lepus nigricollis* from Pakistan. *Journal of Basic and Applied Scientific Research, 3*(11), 339–346.

53. Srebočan, E., Prevendar Crnić, A., Ekert-Kabalin, A.M., Lazarus, M., Jurasović, J., Tomljanović, K., Andreić, D., Strunjak Perović, I. & Čož-Rakovac, R. (2011). Cadmium, Lead, and Mercury Concentrations in Tissues of Roe Deer (*Capreolus capreolus* L.) and Wild Boar (*Sus scrofa* L.) from Lowland Croatia. *Czech Journal of Animal Science, 29*(6), 624–633. https://doi.org/10.17221/249/2010-CJFS

54. Škrivanko, M., Hadžiosmanović, M., Cvrtila, Ž., Zdolec, N., Filipović, I., Kozačinski, L., Florijančić, T. & Bošković, I. (2008). The Hygiene and Quality of Hare Meat (*Lepus europaeus* Pallas) from Eastern Croatia. *Archiv für Lebensmittelhygiene, 59*, 180–184. https://doi.org/10.2376/0003-925X-59-180

55. Špirić, Z., Srebočan, E. & Prevendar Crnić, A. (2012). Mercury in Hares Organs (*Lepus europaeus* Pallas) in the Vicinity of the Mercury-Contaminated Natural Gas Treatment Plant in Croatia. *Journal of Environmental Science and Health A, 47*(1), 77–83. https://doi.org/10.1080/10934529.2012.629584

56. Tariq, S.R., Shafiq, M. & Chotana, A.G. (2016). Distribution of Heavy Metals in the Soils Associated with the Commonly Used Pesticides in Cotton Fields. *Scientifica 2016*(1), 1-11. https://doi.org/10.1155/2016/7575239

57. Tataruch, F., & Kierdorf, H. (2003). Mammals as Biomonitors. In AA Markert, AM Breure, HG Zechmeister (eds) *Bioindicators and Biomonitors* (737–772). Amsterdam: Elsevier.

58. Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. & Sutton, D.J. (2012). Heavy Metal Toxicity and the Environment. *Molecular, Clinical and Environmental Toxicology, 101*, 133-164. https://doi.org/10.1007/978-3-7643-8340-4_6

59. Toman, R., & Massányi, P. (1996). Cadmium in Selected Organs of Fallow Deer (*Dama dama*), Sheep (*Ovis aries*), Brown Hare (*Lepus europaeus*) and Rabbit (*Oryctolagus cuniculus*) in Slovakia. *Journal of Environmental Science and Health A 31*(5), 1043–1051. https://doi.org/10.1080/10934529609376406

60. Tomić Linšak, D., Linšak, Z., Špirić, Z., Srebočan, E., Glad, M., Cenov, A., Jakovac, H. & Milin, Č. (2014). Influence of Cadmium on Metallothionein in Expression and Products of Lipid Peroxidation in the Organs of Hares (*Lepus europaeus* Pallas). *Journal of Applied Toxicology, 34*(3), 289–295. https://doi.org/10.1002/jat.2880

61. Valko, M., Morris, H. & Cronin, M.T. (2005). Metals, Toxicity and Oxidative Stress. *Current Medicinal Chemistry, 12*(10), 1161–1208. https://doi.org/10.2174/0929867053764635

62. Wajdzik, M. (2006). Contents of Cadmium and Lead in Liver, Kidneys, and Blood of the European Hare (*Lepus europaeus*) in Małopolska. *Acta Scientiarum Polonorum. Silvarum Colendarum Ratio et Industria Lignaria, 5*(2), 135–146.

63. Wajdzik, M.., Halecki, W., Kalarus, K., Gąsiorek, M. & Pająk, M. (2017). Relationship between Heavy Metal Accumulation and Morphometric Parameters in European Hare (*Lepus europaeus*) Inhabiting Various Types of Landscapes in Southern Poland. *Ecotoxicology and Environmental Safety, 145*, 16–23. https://doi.org/10.1016/j.ecoenv.2017.06.070

64. Vareda, J. P., Valente, A. J. M. & Durães, L. (2019) Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: A review. *Journal of Environmental Management, 246,* 101-118. https://doi.org/10.1016/j.jenvman.2019.05.126.

65. Venäläinen, E.R., Niemi, A. & Hirvi, T. (1996). Heavy Metals in Tissues of Hares in Finland, 1980–82 and 1992–93. *Bulletin of Environmental Contamination and Toxicology, 56*(2), 251–258. https://doi.org/10.1007/s001289900038

66. Wren, C.D. (1986). Mammals as Biological Monitors of Environmental Metal Levels. *Environmental Monitoring and Assessment, 6*, 127–144. https://doi.org/10.1007/BF00395625

67. Wu, Y., Li, X., Yu, L., Wang, T., Wang, J. & Liu, T. (2022) Review of soil heavy metal pollution in China: Spatial distribution, primary sources, and remediation alternatives. *Resources, Conservation and Recycling, 181,*106261. https://doi.org/10.1016/j.resconrec.2022.106261.

68. Yang, Q., Li, Z., Lu, X., Duan, Q., Huang, L. & Bi, J. (2018) A review of soil heavy metal pollution from industrial and agricultural regions in China: Pollution and risk assessment. *Science of The Total Environment, 642,* 690-700. https://doi.org/10.1016/j.scitotenv.2018.06.068

69. Yoo, Y.C., Lee, S.K., Yang, J.Y., Kim, K.W., Lee, S.Y., Oh, S.M. & Chung, K.H. (2002). Interrelationship between the Concentration of Toxic and Essential Elements in Korean Tissues. *Journal of health science, 48(2)*, 195–200. https://doi.org/10.1248/jhs.48.195

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

(Received on February 2, 2023; accepted on October 10, 2023 – *Primljeno 2. veljače 2023.; prihvaćeno 10. listopada 2023.*)