Environmental cadmium and zinc concentrations in liver and kidney of european hare from different serbian regions

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Abstract

The assayed hares (*n* = 84) were divided into five age groups: 3–6, 12, 12–24, 24–36 and 36+ months. Between all sampling regions (11) significant differences of Cd levels were found in kidney and liver (*p* values of 0.001 and 0.007, respectively). Significant statistical differences (*p* = 0.001) are registered between Cd content in the kidney and liver of hares among all represented age groups. Looking at all investigated hare samples, moderately higher concentrations of Zn were found in the liver (median value: 25.4 mg/kg w.w.) compared to those in the kidney (21.4 mg/kg). These differences were statistically significant (*p* = 0.001). Zinc concentrations in the liver, between all age groups, did not differ significantly (*p* = 0.512) but in the kidney these differences were statistically significant (*p* = = 0.001). Significant differences between Zn concentrations in liver in comparison to kidney (pairwise differences) were found within every single age group with the exception of the oldest (36+). Strong statistically significant correlations (*Ps* – Pearson's correlation) between Cd concentrations in kidney and liver were registered in three groups older than 12 months (*Ps* = 0.81, *p* = 0.001; *Ps* = 0.78, *p* = 0.001 and *Ps* = 0.79, *p* = 0.001, respectively). Negative correlation between Zn and Cd concentrations were found in liver samples within the age group of 12 months (*Ps* = –0.67, *p* = 0.004).

Keywords: cadmium, zinc, kidney, liver, hare.

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One of the major mechanisms of metal input to plants and soils is atmospheric deposition (for example in the forest ecosystems) while anthropogenic sources include agriculture (fertilizers, animal manures, pesticides), metallurgy (mining, smelting and metal finishing), energy production (power plants) and sewage sludge and scrap disposal [1]. Agricultural intensification results in increased mechanization and agro-chemical use, and changes in habitats such as a reduction in diversity [2]. Phosphate fertilizers are known to contain varying levels of heavy metals such as cadmium, lead, nickel and chromium [3]. Cadmium (Cd) and zinc (Zn) are elements that have similar geochemical and environmental properties [4,5]. The chemically and physically similar but essential element zinc (Zn) is also strongly enriched in precipitation over different areas [6]. The co-occurrence of these two metals in the natural environment and their possible interactions in biological systems are therefore of particular interest.

Toxicity in wildlife from metals exposures is generally poorly understood and is rarely quantified in field settings. Animal tissue levels can provide important data regarding the fate and bioavailability of heavy metals within natural ecosystems [7–9]. In general, the gastrointestinal tract and the liver regulate the uptake and transfer of Zn. Interactions between essential and non-essential metals are very common (*e.g.*, Cd uptake can mimic that of Zn).

The objectives of this study were to evaluate the environmental Cd and Zn concentrations in European hare from different Serbian regions. The tissue samples acquired bring up a concept of using hares as promising Cd and Zn biomonitors as well as to investigate how the different age distribution within hare population affects comparison between Cd and Zn levels among sampling regions and age groups. The present study was also projected to estimate bioaccumulation trends of Cd and Zn during the lifetime of European hares and interactions between Cd and Zn in hare organs if any exists and try to model the dependency of liver Cd and Zn concentrations.

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EXPERIMENTAL

Materials and methods

A total of 168 tissue samples (84 kidneys and 84 livers) obtained from all hunted free-range hares (*Lepus europaeus*) were investigated for Cd and Zn presence. The hares were acquired from eleven regions of western, central and southern parts of Serbia during regular hunting season 2010/2011. The geographical locations from which samples had been collected are shown in Figure 1.

The eye samples were used for estimating the age of the hares. The weight of the eye lens increases because of insoluble proteins that accumulate in it and this process correlates with the animal's age. After extraction, eye samples were placed in marked plastic bags with zipper open/close system, and immediately transported to the laboratory. The eye lenses were fixed in 5% formalin for 72 h and then dried at 37 °C for 96 h, under normal pressure. After they were dried, the lenses were weighed on a precise analytical scale (Mettler AE 200) to 1 mg precision. For further data analysis, the hares were sub-divided according to their age into five groups: 3–6 months old (100–200 mg), 12 months old (200–280 mg), 12–24 months old (280–310 mg); 24–36 months old (310–370 mg) and older than 36 months (≥ 370 mg).

The whole liver and kidney were sampled from each animal. The liver and kidney samples were stored at –20 °C until analysis. After homogenization, tissue samples (1 g) were digested with 8 ml of HNO_3 (65% v/v, analytical grade, JT Baker, Center Valley, USA) and 2 ml of H_2O_2 (30%, analytical grade, Kemika, Zagreb, Croatia) using the method of acid microwave digestion. The samples were digested in a microwave digestion unit (Milestone TC, EVISA, EU) with temperature control. The digestion program began at a potency of 1000 W, then it was ramped for 10 min to 200 °C, after which the samples were held at 1000 W and a temperature of 180 °C for 20 min. Calibration standards were prepared from commercial solutions in HNO_3 (0.2%) with 1.000 mg/l of each element (JT Baker, Center Valley, PA, USA). All results are expressed on wet weight basis (w/w).

Cadmium concentrations were determined by the AAS graphite furnace technique at 228.8 nm using a Varian SpectrAA 220 atomic absorption spectrophotometer, equipped with a Varian GTA 110 furnace with constant temperature zone. Zinc concentrations were measured by flame atomic absorption spectrophotometry (FAAS) at 213.9 nm with deuterium background correction. The maximum allowable relative standard deviation between three replicates was set to 5%. The trueness of the method was tested with standard reference material – pig kidney (BCR No.186) from the Community Bureau of Reference and recoveries. Cd in standard reference material deviated at most by ±10% from the certified mean values, whereas Zn deviated at

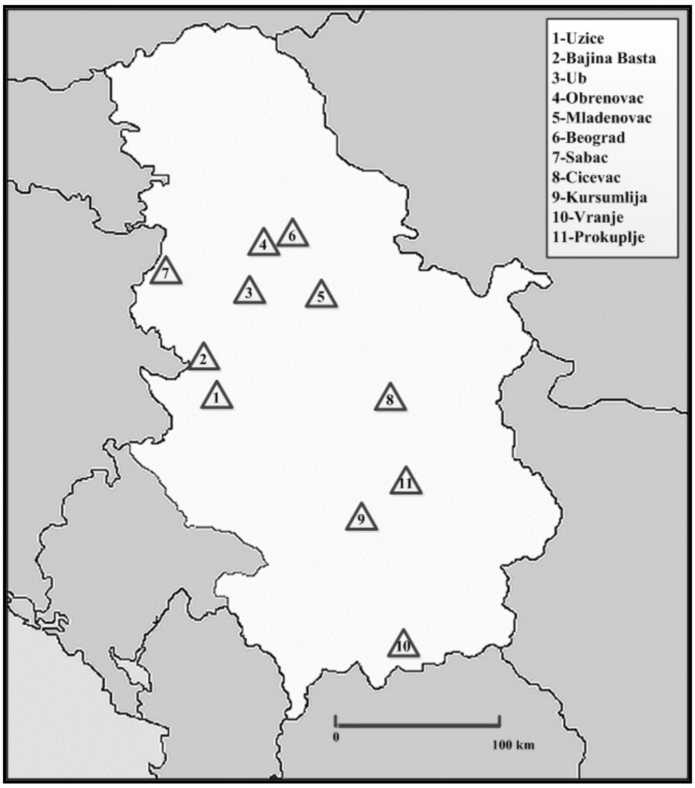

Figure 1. Map of sampling regions.

most by +8%. The recovery of Cd and Zn was determined by adding a known amount of a particular standard solution into the samples. Recoveries of added Cd and Zn standards in the analyses were controlled in randomly selected samples, and fell within the range of 95–102%. The detection limits for Cd and Zn were 0.005 and 0.2 mg/kg, respectively.

Data analysis

Statistical analysis was performed using the MINITAB software package, version 16.0. Concentrations were expressed as median values and range of minimum to maximum. The Kruskal-Wallis test and the post-hoc Man-Whitney non parametric test were used to examine statistical differences of heavy metal concentrations among groups. The Wilcoxon signed rank test was used to examine differences between Cd and Zn concentrations in kidney and liver within age groups. The significance of correlations between Cd and Zn levels were calculated using Pearson's correlation (*Ps*). The differences were considered statistically significant when the *p* value was less than 0.05.

RESULTS AND DISCUSSION

Accumulation of toxic and essential elements in hare organs has been studied by a number of authors [10–15]. Considering values obtained by sampling regions (Table 1) we noticed that the median values, of both the metals, are probably affected by random individual variations, age structure of collected animals and the sample size from the particular sampling region.

The concentrations of Cd and Zn in brown hare organs in relation to sampling regions are listed in Table 1.

Looking at all sampling regions (Figure 1) significant differences of median values were noted in Cd levels in kidney and liver ($p = 0.001$ and $p = 0.007$, respectively). Significant statistical differences (p=0.001) were registered between Cd content in the kidney and in the liver ($p = 0.001$) of hares among all represented age groups. Age trends of Cd and Zn concentrations in various organs of European hare are shown in Table 2.

For Zn, within the investigated hare samples ($n = 84$), higher concentrations (expressed as median values) were found in liver (25.4 mg/kg w.w.) and slightly lower Zn concentrations (21.4 mg/kg w.w.) were found in kidney samples. These differences were statistically significant ($p = 0.001$).

Based on non-parametric analysis between sampling localities, we found significant differences of Zn concentrations in the kidney ($p = 0.001$) while in the liver these differences were not statistically siginificant ($p = 0.155$). Zinc concentrations in the liver, between all

Table 1. Metal concentrations (mg/kg w.w.) in kidney and liver of hares from different Serbian sampling regions (n=84)

Region	*n*		Cd		Zn	
			Kidney	Liver	Kidney	Liver
1-Uzice	10	Median	1.65	0.13	23.2	25.8
		Range	0.27–3.10	0.06–0.17	20.5–30.8	21.3–31.7
2-Bajina Basta	6	Median	1.85	0.11	21.8	25.0
		Range	0.38–7.54	0.01–0.85	19.3–31.5	18.6–26.4
3-Ub	10	Median	1.96	0.12	24.0	25.6
		Range	0.64–4.97	0.05–0.45	17.8–37.0	17.3–33.5
4-Obrenovac	6	Median	3.15	0.26	22.6	25.5
		Range	0.18–5.12	0.05–0.32	17.8–25.7	22.5–32.7
5-Mladenovac	10	Median	1.33	0.11	18.2	24.7
		Range	0.15–2.97	0.02–0.33	14.1–24.2	18.7–28.0
6-Beograd	7	Median	1.63	0.24	21.3	21.8
		Range	0.49–5.36	0.08–0.32	18.0–22.2	15.5–26.9
7-Sabac	9	Median	2.05	0.14	16.8	20.9
		Range	0.66–5.30	0.04–0.35	16.0–26.6	17.8–32.2
8-Cicevac	7	Median	2.39	0.25	22.2	24.8
		Range	1.83–3.10	0.17–0.29	19.6–23.8	22.0–28.8
9-Kursumlija	6	Median	0.96	0.04	16.4	26.6
		Range	0.16–2.00	0.01–0.14	13.9–21.6	23.6–27.7
10-Vranje	6	Median	3.73	0.28	22.4	29.0
		Range	0.53–5.10	0.08–0.70	17.9–22.9	19.5–33.5
11-Prokuplje	7	Median	0.32	0.07	18.3	25.4
		Range	0.09–0.95	0.02–0.23	12.6–20.4	22.9–31.1

Table 2. Cd and Zn content (mg/kg w.w.) in kidney and liver by age groups

Age, months	*n*		Cd		Zn	
			Kidney	Liver	Kidney	Liver
3–6	11	Median	0.32	0.05	17.2	24.6
		Range	0.15–0.71	0.01–0.24	12.6–30.8	17.8–31.1
12	16	Median	0.93	0.09	19.8	25.1
		Range	0.21–2.21	0.02–0.31	16.0–24.7	15.9–27.1
12–24	17	Median	1.78	0.14	22.1	25.7
		Range	0.71–2.97	0.09–0.33	15.6–26.8	18.6–31.7
24–36	28	Median	2.8	0.26	22.2	24.9
		Range	1.83–4.97	0.07–0.70	16.2–24.8	15.5–33.5
36+	12	Median	4.91	0.32	23.7	25.8
		Range	3.08–7.84	0.17–0.85	16.8–37.1	21.8–32.1

age groups, did not differ significantly ($p = 0.512$) but in the kidney these differences were statistically significant ($p = 0.001$).

Pairwise differences between Zn concentrations in liver and kidney within every single age group are given in Table 3.

*Table 3. Pairwise differences of Zn content in liver and kidney within age groups; * – statistically significant differences (p < 0.05)*

Age, months	*p* Value
3–6	0.001*
12	0.002*
12–24	0.015*
24–36	0.002*
36+	0.926

The significant correlation between Cd and Zn concentrations in the kidney (CdK-ZnK) within the investigated hare samples and in different tissue samples are presented in Table 4.

Strong statistically significant correlations between Cd concentrations in kidney and liver were found in three groups older than 12 months. Negative correlation ZnL-CdL was found in the liver within the age group of 12 months.

However, changes in the slope constant Zn/Cd for the liver samples, sorted by age, may reflect environmental Cd exposure during the individual development of European hares (Figure 2).

Looking at the slope constants Zn/Cd among age groups presented in Figure 2, in a form of linear regression equation $Y_{ZnL} = kX_{CdL} + b$ (ZnL – zinc concentration in liver; CdL – cadmium concentration in liver; b – intercept value with *Y* axis), we registered a sharp decline of the regression line ($k = -30.1$) in age group of 12 months. This trend is also supported by taking into account the strong negative correlation found within this age group. It seems that Cd amplifies Zn deficiency in yearlings but also reduces or delays toxic effects of Cd at presented levels. The significant correlations of Cd concentration in different tissue (CdK-CdL) are registered in age groups older than 12 months (12–24 months: $Ps = 0.81$, $p = 0.01$; 24–36 months: $Ps = 0.78$, $p = 0.001$; ≥36 months: $Ps = 0.79$, $p = 0.004$). These correlations were not registered in age groups 3–6 and 12 months ($Ps = 0.142$; $p = 0.552$ and $Ps = 0.06$; $p = 0.826$, respectively). Further, the slope constants Zn/Cd given in Figure 2 arise in subsequent age groups in order: -5.33, –1.6 and 3.1. Such difference between bioaccumulation rates of Zn and Cd in the liver can be used as an indicator of Cd exposure [16]. In principle, there has been a distinct increase of bioaccumulation

*Table 4. Significant correlations between and within tissue metal concentrations (in all investigated samples and by age groups); Ps – Pearson's correlation coefficient; * – statistically significant correlations (p < 0.05); r² – coefficient of determination; CdK – cadmium in kidney; ZnK – zinc in kidney; CdL – cadmium in liver; ZnL – zinc in liver; 12–24; 24–36; 36+ (age groups)*

	CdK	CdL
ZnK	$Ps = 0.57$; $p = 0.001$*; $r^2 = 0.32$	$Ps = 0.52$; $p = 0.001$*; $r^2 = 0.27$
CdL	$Ps=0.81$; $p=0.001$*; $r^2 = 0.70$	–
ZnL	–	$Ps = -0.67$; $p=0.004$*; $r^2 = 0.46$

	$CdK_{12–24}$	$CdK_{24–36}$	CdK_{36+}
CdL_{12-24}	$Ps = 0.81$; $p = 0.001$*	–	–
CdL_{24-36}	–	$Ps = 0.78$; $p = 0.001$*	–
CdL_{36+}	–	–	$Ps = 0.79$; $p = 0.004$*

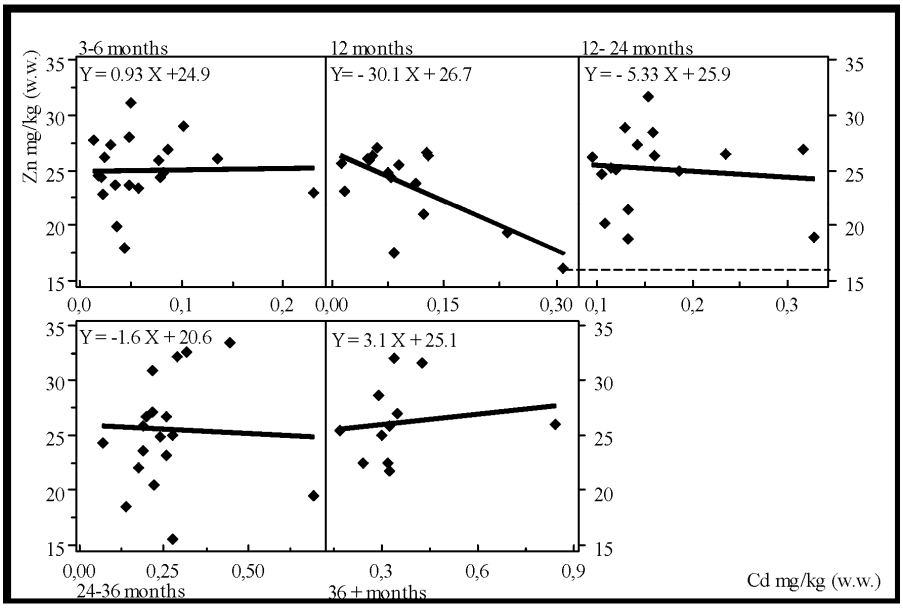

Figure 2. Relationship between Zn and Cd concentrations in liver with regression line by age groups; Y-axis: Zn concentrations in liver; X-axis: Cd concentrations in liver.

of Cd in hare organs during subsequent stages of life (Table 2). A somewhat higher increase of hepatic Zn related to Cd was registered in the oldest age group (≥ 36 months). It can be interpreted that Zn, as an essential element, has a homeostatic mechanism that maintains optimum tissue levels over a range of exposure to environmental Cd. It can also be speculated, considering the obtained results of hepatic and renal Zn concentrations in yearlings, that Cd is simply transferred to metallothionein (MT) according to their binding affinity with subsequent displacement of Zn [17].

Intercepts calculated form equations (Figure 2) related to hepatic Zn-Cd correlations by age groups are: 24.9, 26.7, 25.9, 20.6 and 25.1, respectively. It can be stated that these values may correspond to the physiological concentrations in the liver of the hares studied. Registered background tissue levels of Zn refers to those concentrations of metals that derive from natural as well as anthropogenic sources that are not the focus of the risk assessment. Distinct age trends of Zn concentration in liver of European hare have not been established. It is probably because of the native liver MTs of most animals predominantly contain Zn bounded tightly, and are less able to be substituted by Cd.

The relationship with respect to Zn and Cd concentrations in liver and kidney within all investigated hare samples are given in Figure 3.

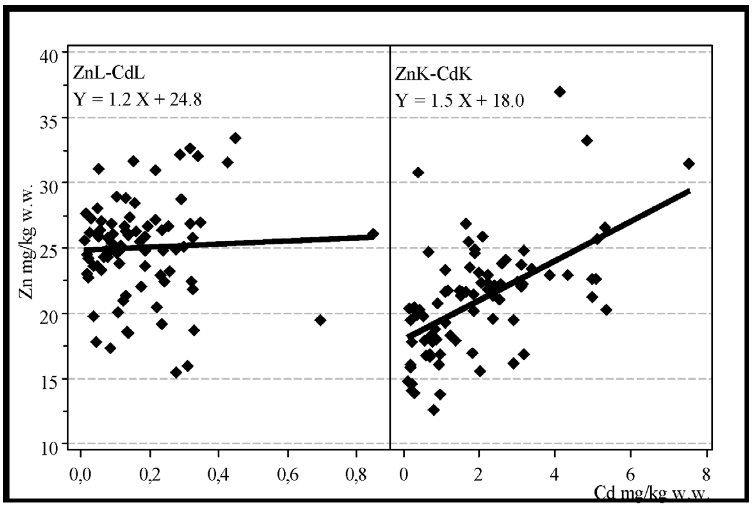

Figure 3. Relationship between Zn and Cd concentrations in liver and kidney with regression line within all investigated hare samples (n = 84); ZnL-CdL: scatter plot with regression line and fitted intercept of Zn and Cd concentrations in liver; ZnK-CdK: scatter plot with regression line and fitted intercept of Zn and Cd concentrations in kidney.

The increase of Zn content with elevated Cd concentrations in the kidney, looking at the investigated hare samples, was more distinct in comparison to the liver (Figure 3). However, the reason why the less distinct increase of Zn in relation to Cd at high concentrations in the same organ is unknown at present.

The difference between particular values of Zn in liver probably results from the different sex, age, diet and inhabitation conditions. Although the hare relies largely on grasses for food, its diet composition may vary markedly from one area to the next [18]. Under stressful conditions, hares consume increased quantities of browse and plant biomass of very low nutritional value, such as bark, pine needles, etc. [19,20]. Seasonal variations in the diet may vary from periods when animals eat more plants with wood stems or when they eat more grass. The grass regenerates yearly, whereas, for example, willow and birch are exposed to the influence of air pollution for longer periods, during which they accumulate heavy metals.

CONCLUSIONS

It can be noted that the biological role of Zn metabolism during development and growth of European hare is very important. Considering the concentrations of Cd, during the individual development of European hare, it should be stated that there is a distinct increase of bioaccumulation of Cd during subsequent stages of life. Observing the relationship between Cd and Zn levels within various age groups, it can be concluded that the bioaccumulation process comes after the first year of life. It may be important information if the hare organs are intended to be used for environmental biomonitoring of Cd. Furthermore, age distribution suggests that the samples can be censored for age to include those of animals with an exposure period of 2 or 3 years, collected from the regions of interest, and the obtained results compared with yearlings. Such age censoring would increase the monitoring precision in sampling a specific exposure period in a long-term monitoring program. Metals uptake, therefore, likely reflects metals availability through diets based on the composition and structure of specific ecosystems as affected by current stressors. Observed animals inhabiting the studied areas in Serbia show similar to lower Cd and Zn bioaccumulation compared to other biotopes in Europe. The strong age dependency due to Cd accumulation in hare organs precludes direct comparison of different groups (areas etc.), unless the age distributions are fairly equal.

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IZVOD

SADRŽAJ KADMIJUMA I CINKA IZ ŽIVOTNE SREDINE U JETRI I BUBREZIMA DIVLJIH ZEČEVA SA RAZLIČITIH PODRUČJA SRBIJE

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(Naučni rad)

Ukupno je ispitano 168 uzoraka tkiva (jetra i bubrezi) sa 84 divlja zeca sakupljenih iz 11 različitih područja Srbije na prisustvo kadmijuma (Cd) i cinka (Zn). Jaka statistička povezanost između količina kadmijuma registrovanih u bubrezima i jetri je registrovana kod životinja starijih od 12 meseci, što ukazuje na prisutnost ovog metala u njihovom okruženju. Značanje statističke razlike između koncentracija cinka u jetri u odnosu na bubrege su utvrđene unutar svih prisutnih starosnih grupa, izuzimajući najstariju. Negativna korelacija (*Ps* - Pirsonov korelacioni koeficijent) je registrovana unutar starosne grupe od 12 meseci ($Ps = –0,67$, $p = 0.004$). Utvrđeno je da cink kod ispitane populacije divljeg zeca pokazuje homeostatski mehanizam koji u prisustvu toksičnog elementa, kao što je kadmijum, održava optimalni nivo ovog esencijalnog elementa u ispitanim tkivima. Uočeno je da se sadržaj cinka, izražen kao vrednost medijane u jetri po starosnoj dobi, ne menja značajno dok je u bubregu u blagom porastu, mada su individualne varijacije jako prisutne. Izmerene vrednosti cinka u ciljnom organu – jetri se nalaze u okviru normalnih vrednosti za hepatično tkivo. Pouzdano je utvrđeno da su medijan vrednosti ispitanih metala pod jakim uticajem starosne strukture uzoraka divljeg zeca kao i regionalnih razlika u prisutnosti kadmijuma i cinka u životnoj sredini. Utvrđen je odličan biomonitorski potencijal tkiva divljeg zeca za sistemski monitoring i praćenje aerodepozicije kadmijuma obzirom na način ishrane, životni vek, radijus kretanja, težinu, adaptibilnost, dostupnost i pokrivenost u velikom broju staništa Srbije, uključujući i oblasti u neposrednoj blizini zagađivača (termoelektrane, pepelišta, površinski kopovi uglja, rafinerije) i poljoprivrednih područja sa raširenom primenom fosfatnih đubriva i agrohemikalija.

Ključne reči: Kadmijum • Cink • Bubreg • Jetra • Divlji zec