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Original article

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

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Abstract

The study aimed to determine the content of selenium (Se), zinc (Zn), copper (Cu) and cadmium (Cd) in the liver of predominantly plant-eating omnivore wild boar (Sus scrofa), predominantly meat-eating omnivore red fox (Vulpes vulpes) and herbivore red deer (Cervus elaphus), from North-Eastern Poland (Warmia and Mazury), in order to verify the distribution of these elements in the trophic pyramid. Furthermore, the study was used to assess the risk of eating venison. Samples were analyzed using atomic absorption spectrophotometry. The average concentration of Se was 3.9 ($p<0.001$) and 1.8-fold higher ($p<0.001$) in the wild boar and red fox, respectively, in comparison to the red deer, and 2.1-fold higher in the wild boar comparing to the red fox ($p<0.001$). There was no difference in the average concentration of Zn. The average concentration of Cu was 9.3. Concentration of this element was 5.4-fold higher in red deer in comparison to red fox ($p<0.001$) and 9,34-fold higher than in wild boar ($p<0.001$).

The average concentration of Cd was 1.9-fold higher in wild boar in comparison to the red fox ($p<0.029$). Correlation between Cu and Cd concentrations was also observed in the case of the red deer and red fox, while no such correlations were observed between the tested elements in the wild boar. In conclusion, the liver concentrations of these heavy metals in selected wild animas species from the hunting areas of Warmia and Mazury, do not exceed standard safe values for consumers. Moreover, the wild red deer population in North-Eastern Poland is significantly Se deficient.

Key words: cadmium, copper, hepatic tissue, wild animals, selenium, zinc

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Introduction

Due to the increasing venison consumption around the world, the monitoring of micronutrients and heavy metal concentrations in the tissues of wild animals seems to be significant. Several matters, including the definition of reference values of heavy metals in wild animal tissues, possible adverse effects of heavy metals for the consumers, as well as the nutritional value of venison, need to be addressed.

Heavy metals are naturally present in the environment. For many decades forest areas of Central Europe have been exposed to heavy metal pollution that comes from industry, transport, and other human activities (Kuiters 1996). When soil pH decreases due to acid rain, the solubility and bioavailability of metals, i.e., cadmium (Cd), increase (Medvedev et al. 1997). Heavy metals content in tissues of plants and herbivorous animals also increases (Falandysz et al. 2015). Venison is a reliable indicator of environmental pollution with heavy metals. Due to the relatively long lifespan of wild animals, the accumulation of heavy metals in their tissues is more prolonged (Suran et al. 2013).

Cadmium is considered one of the most dangerous heavy metals that pollutes the environment (Haider et al. 2015). This element can be one of the etiological factors of many pathological processes in the body, such as changes in ovarian structure, kidney function disorders, growth inhibition, changes in the central nervous system, liver dysfunction, and bronchitis. It also exhibits strong teratogenic effects (Rous and Jelinek 2000, Paniagua-Castro et al. 2007).

Copper (Cu) plays a role in the regulation of many biochemical processes. It is a component of several enzymes, including superoxide dismutase, ceruloplasmin, lysyl oxidase, cytochrome oxidase, tyrosinase, and affects the oxidative balance of the body by showing antioxidant activity (Abdelghany and Elkhaiat 2015, Wysocka et al. 2019).

Zinc (Zn) is a component of nearly 300 enzymes and plays various functions in the body (Miao et al. 2013, Prasad 2013). It affects the immune response, the expression of lymphocyte genes, development, and activity of neutrophils and NK cells (natural killer cells) (Kincaid 1999). Although Zn and Cu are necessary for the proper functioning of the body, they can cause symptoms of poisoning, when administered in too high amounts (Gaetke and Chow 2003).

Selenium (Se) is a constituent of nearly 30 selenoproteins, most of which are enzymes. Pathological conditions associated with Se poisoning are mainly manifested by skin lesions such as baldness and hoof necrosis (Whanger et al. 1996).

Game meat and venison are consumed in Poland and neighboring countries. Thus, it is crucial to ensure the control of heavy metal contaminants in meat and offal and assess the risk of their consumption. One of the most frequently encountered free-living, wild animals in Poland are wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), and red fox *(Vulpes vulpes)* (CSO 2015). Therefore, it seems understandable that scientists take much interest in research on those species as bioindicators of heavy metal pollution.

This study aimed to determine the content of Se, Zn, Cu and Cd in the liver of predominantly plant-eating omnivore wild boar (*Sus scrofa*), predominantly meat-eating omnivore red fox (*Vulpes vulpes*) and herbivore red deer (*Cervus elaphus*), from North-Eastern Poland, in order to verify the distribution of these elements in the trophic pyramid. Furthermore, the study was designed to assess the risk of eating venison.

Materials and Methods

The research was carried out on three species of wild mammals – the wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*) and red deer (*Cervus elaphus*) from the region of Warmia and Mazury. The research area included 10,000 square kilometers of North-Eastern Poland (53.78°N, 20.49°E, 139 m above sea level).

The research was conducted during the hunting season from October to December 2015. Tissue samples were obtained from ten red deer, ten wild boars, and ten red foxes. The animals were shot by hunters under the hunting law and did not show any external signs of disease. Liver samples (200g) were collected from the animals immediately after the shot and placed in individual, sterile, plastic containers, and directly sent to the laboratory under refrigeration conditions. Every sample was homogenized using a food processor, and 2 g of homogenized tissue was weighted for further examination (8 g total). Homogenates were incinerated for 12 hours in quartz crucibles in a muffle furnace at 450°C to obtain dry mass. The ash residue was dissolved in concentrated hydrochloric acid, heated, and then deionized water was added up to 10 ml. The concentration of Cu, Zn, Se, Cd was measured in the supernatant by flame atomic absorption spectrometry using AAS Solaar M6. The tests were carried out at the University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic).

Collected data were compared using Python 3.7 software (Python Software Foundation, https://www.python.org/). A descriptive analysis was done with the Pandas library to describe the function. Kruskal-Wallis test for independent samples was performed for Se, Zn, Cu and Cd concentrations in selected species. When this test revealed significant differences, the Mann-Whitney-U test was used for group comparison. The

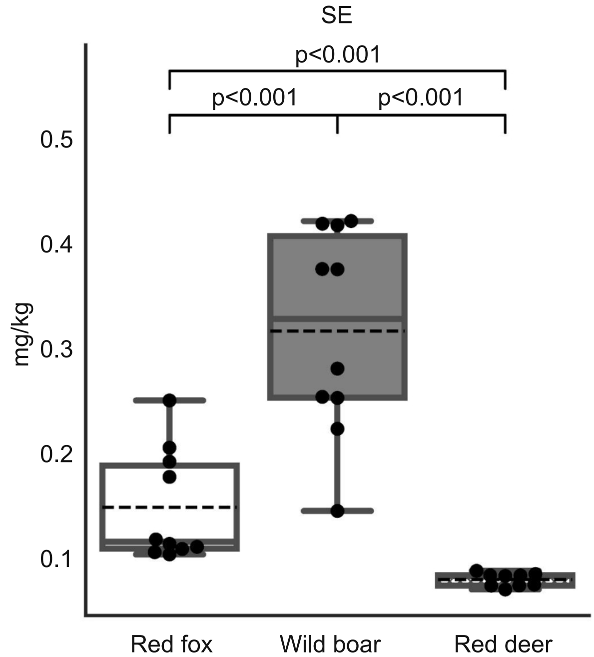

Fig. 1. Concentrations of selenium (Se) in the liver tissue of selected wild animal species.
The boxes extend from the 25th percentile to the 75th percentile; the whiskers represent the lowest and highest concentrations, excluding outliers. The solid line in the center of the box indicates the concentration median. The dashed line in the box indicates the mean concentration. Black markers describe the exact concentration of the samples. Higher p-values of post hoc comparison between animal species corrected by Bonferroni test than 0.001 are presented as an exact value.

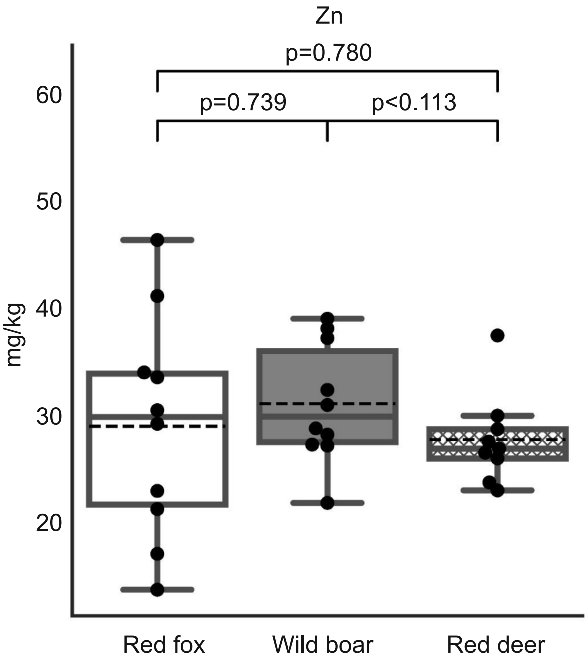

Fig. 2. Concentrations of zinc (Zn) in the liver tissue of selected wild animal species.
The boxes extend from the 25th percentile to the 75th percentile; the whiskers represent the lowest and highest concentrations, excluding outliers. The solid line in the center of the box indicates the concentration median. The dashed line in the box indicates the mean concentration. Black markers describe the exact concentration of the samples. Higher p-values of post hoc comparison between animal species corrected by Bonferroni test than 0.001 are presented as an exact value.

data were presented as a mean ± standard deviation (SD). Spearman's Rank-Order Correlation test was used for the calculation of the correlation coefficient between Se, Zn, Cu and Cd in each species.

Results

The concentrations of the Se, Zn, Cd, and Cu in the liver tissue are presented in Figs. 1, 2, 3, and 4.

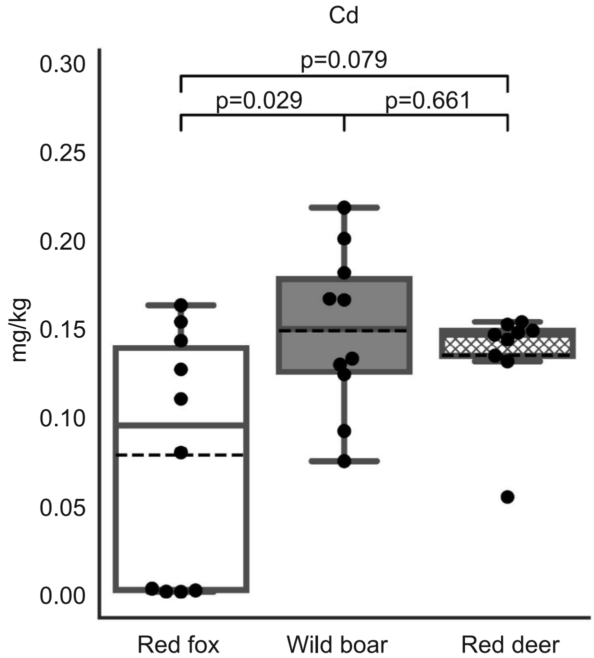

Fig. 3. Concentrations of cadmium (Cd) in the liver tissue of selected wild animal species.
The boxes extend from the 25th percentile to the 75th percentile; the whiskers represent the lowest and highest concentrations, excluding outliers. The solid line in the center of the box indicates the concentration median. The dashed line in the box indicates the mean concentration. Black markers describe the exact concentration of the samples. Higher p-values of post hoc comparison between animal species corrected by Bonferroni test than 0.001 are presented as an exact value.

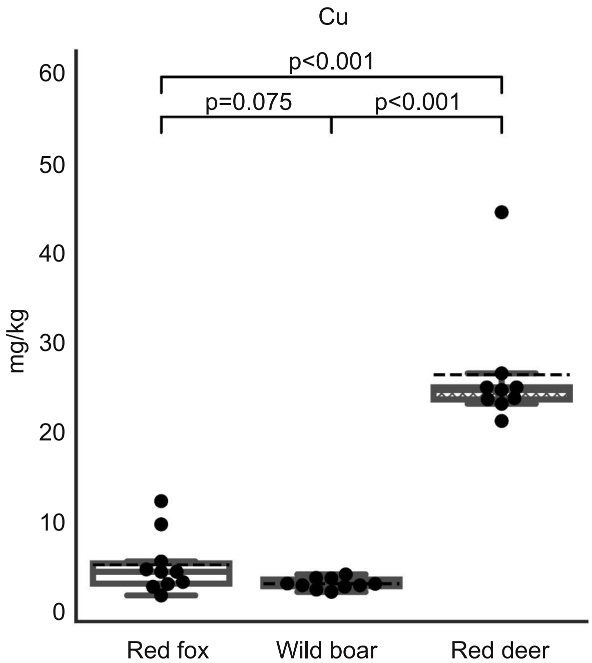

Fig. 4. Concentrations of copper (Cu) in the liver tissue of selected wild animal species.
The boxes extend from the 25th percentile to the 75th percentile; the whiskers represent the lowest and highest concentrations, excluding outliers. The solid line in the center of the box indicates the concentration median. The dashed line in the box indicates the mean concentration. Black markers describe the exact concentration of the samples. Higher p-values of post hoc comparison between animal species corrected by Bonferroni test than 0.001 are presented as an exact value.

The average concentration of Se was 3.9-fold higher in the wild boar (0.32±0.09 mg/kg, $p<0.001$) and 1.8-fold higher in the red fox (0.14±0.05 mg/kg, $p<0.001$) in comparison to the red deer (0.08±0.01 mg/kg) and 2.1-fold higher in wild boar comparing to red fox ($p<0.001$) (Fig. 1).

There was no significant difference in the average concentration of Zn between the red fox (28.86±10.35

Table 1. Spearman's correlation coefficients between the content of Se, Cd, Zn, Cu concentrations in selected species (A – wild boar, B – red dear, C – red fox). The upper diagonal of each subtable presents correlation coefficients. The lower diagonal of each subtable presents an exact level of significance.

A) **Wild boar**	**Se**	**Cd**	**Zn**	**Cu**
Se		-0.042	-0.382	-0.248
Cd	p=0.907		0.115	0.539
Zn	p=0.276	p=0.751		0.309
Cu	p=0.489	p=0.108	p=0.385	

B) **Red deer**	**Se**	**Cd**	**Zn**	**Cu**
Se		-0.300	0.400	-0.100
Cd	p=0.624		0.500	0.900*
Zn	p=0.505	p=0.391		0.800
Cu	p=0.873	p=0.037	p=0.104	

C) **Red fox**	**Se**	**Cd**	**Zn**	**Cu**
Se		-0.055	0.067	0.309
Cd	p=0.881		0.697*	0.733*
Zn	p=0.855	p=0.025		0.879**
Cu	p=0.385	p=0.016	p=0.001	

* $p \leq 0.05$; ** $p \leq 0.001$

mg/kg), the wild boar (30.97±5.57 mg/kg), and the red deer (28.60±1.85 mg/kg) (Fig. 2).

The average concentration of Cd was 1.9-fold higher in the wild boar (0.15±0.05 mg/kg) in comparison to the red fox (0.08±0.06 mg/kg, $p<0.029$). The average Cd level in the red deer was similar to that found in the wild boar - 0.15±0.01 mg/kg (Fig. 3).

The average concentration of Cu was 9.3 and 5.4-fold higher in red deer (27.64±9.51 mg/kg) in comparison to red fox (5.06±3.30 mg/kg, $P<0.001$) and 9,34-fold higher than in wild boar (2.96±0.61 mg/kg, $P<0.001$) (Fig. 4).

There was no significant correlation between Se, Zn, Cd, and Cu in wild boar liver tissue. A strong correlation was found between Cu and Cd in the red deer (0.9, p=0.037). A strong correlation was also observed between Cu and Cd (0.733, p=0.016) and Cu and Zn (0.879, p=0.001). A moderate correlation was observed between Zn and Cd (0.697, p=0.025) (Table 1).

Discussion

The present study carried out in North-Eastern Poland demonstrated that the level of Se was higher in wild boars and foxes in comparison to red deer. It may be related to the fact that these animals are at the top of the food pyramid - their food is based on small animals and plants, which may be the source of this element accumulation (Piskorová et al. 2003).

The Se level in deer was similar to the results obtained by Falandysz et al. (2005), who studied the Se content in the liver of the red deer in North-Eastern Poland. Analogous results were also obtained by Pilarczyk et al. (2008). In contrast, a study conducted in the Norwegian region of Hiltra showed a higher liver concentration of Se in deer (0.13 mg/kg) (Vikøren et al. 2005).

In the area of Warmia and Mazury, the soils are deficient in Se. Therefore, the insufficient intake of this vital microelement is widespread in ruminants (Bednarek and Bik 1994). The value of Se < 0.6 mg/kg in the dry liver matter is considered as deficient (Pollock 2005). In comparison with the population of red deer kept on a farm, with symptoms of white muscle disease, the Se value was lower than 0.12 mg/kg (Grace and Wilson 2001). Given this restrict limit, all deer tested in the present study were profoundly deficient in Se.

The Se level in foxes was at a reasonably high level of 0.14 mg/kg. Studies carried out in Southern Spain have shown that Se concentration in fox was 0.0535 mg/kg (Millan et al. 2008). On the other hand, research conducted on a fox farm in Western Pomerania, Poland, showed a Se concentration of 0.0247 mg/kg (Balicka-Ramisz et al. 2010), which is considered as deficient. Based on the results obtained in the mentioned studies, it can be assumed that foxes used in our research, as omnivores, had an adequate level of Se.

In our research, the Se level in the wild boar was the highest in comparison with that found in the other species (Fig. 1). A similar study carried out on the wild boar in the region of North-Eastern Poland by Pilarczyk et al. (2010) showed the Se concentration of 0.19 mg/kg, which was lower than the level revealed in our study. Unfortunately, there are no reference values for Se level in the wild boar. Because of that, we used the standards for pigs. According to Puls (1994), criteria used in the diagnosis of Se deficiency in the swine liver are as follows: below 0.11 µg/g is deficiency, 0.12-0.39 µg/g is marginal level, above 0.40 µg/g is optimal level for animals. Considering these criteria, the Se level in wild boar should be classified as evidence of Se deficiency.

The level of Se in the liver depends on the species predisposition, form, and distribution of Se in feed, as well as the individual body's abilities. Equally important, especially for ruminants, is the presence of Se in the environment – soil and plants. Legumes have less capacity to accumulate this element than grasses. Red deer have a lower level of Se absorption compared to wild boars or foxes. This may be accorded to the fact that in ruminants, Se after absorption by rumen microbes is absorbed in the small intestine, cecum, and colon. Diet rich in small-molecule proteins, vitamins A, C, and E increases the bioavailability of this element. Oppositely Cd, Hg, and As have a negative effect on Se absorption.

In animals of the Suidae family, organic Se is absorbed at the level of 77%. On the other hand, in ruminants, only 29% of Se is absorbed as a result of the activity of rumen microbes (Meschy 2010). The Se bioavailability also depends on the form and composition of the feed. The level of Se bioavailability in monogastric animals is up to 80%, while in ruminants it does not exceed 51% (Koenig et al. 1997). Young ruminants absorb more Se, due to the still undeveloped function of the rumen. Se has an influence on the brain function, endocrine glands, and reproductive organs (Chen and Berry 2003). These organs have priority in the absorption of this element before the liver, heart, skeletal muscles, and erythrocytes. Wild boars are monogastric and compared to ruminants, the Se absorption level in these animal is higher (Fig. 1).

Zn is essential for the functioning of many enzymes and is necessary for growth and reproduction. In our study, the lowest level of this element was determined in red deer. Compared to studies conducted in Lower Silesia (Poland), the concentration of Zn in the hair of the red deer was between 114 and 158 mg/kg, and in the hair of the wild boar was 44.57 mg/kg. A lower concentration of Zn was observed in the animal parenchymatous organs (28.46 mg/kg) (Cygan-Szczegielniak et al. 2018). In Spain, the average level of Zn in the liver of the red fox was 77 µg/g (Pérez-López et al. 2015), while in the arctic fox was 16.0 mg/kg (Heltai and Markov 2012). The present study has revealed that the liver concentration of Zn in the red deer and red fox from Warmia and Mazury is lower than in Spain, higher than in the Arctic, but similar to other parts of Poland.

In this research, the level of Cu in the wild boar and red fox liver tissue was lower than that determined in the wild boar liver tissue in Italy (46.12, mg/kg) (Amici et al. 2012) and red fox liver tissue from an area of "Sarnena Sredna Gora" mountain in Bulgaria (15.121 mg/kg) (Georgiev et al. 2018). The level of Cu in the red deer liver tissue from Warmia and Mazury was higher than that found in the red deer from western Norway (20 µg/g) (Vikøren et al. 2005).

Cu deficiency is a common problem in all ungulates, including deer. It can be of a primary or secondary character. The primary is characteristic for meadow and swampy areas. The secondary is observed when the Cu level in feed is inadequate. Cu deficiency may cause many diseases, for example, enzootic ataxia (Brightling 1983, Whitelaw 1985, Handeland et al. 2000). Geographical variation in Cu concentration in red deer probably reflects the difference in Cu levels in plants, indicating the soil level of bioavailable Cu. The Cu content in plants is also dependent on the content of Cu inhibitors, such as Zn or Cd in soil. Cu deficiency causes growth disturbances and is easy to overlook in the wild animal populations (Underwood and Suttle 1999). The most sensitive to Cu deficiency are young, rapidly growing animals, animals in the first pregnancy, or during lactation (Hosking et al. 1986).

Cd is naturally present in the environment in small quantities. However, as a result of human activity, mostly in the industry and transport sectors, its levels increase. When the soil pH decreases due to acid rain, the solubility and availability of Cd rise. In the present study, the highest level of Cd was in the red deer and wild boar and the lowest in the red fox liver tissue.

In comparison with results obtained in Spain (Santiago et al. 1998), we determined lower Cd concentration in the liver of the red deer. The higher level of Cd in comparison to that observed in populations living in Warmia and Mazury was also found in the liver of the Eurasian wild boar and the red deer in North-Eastern Croatia. The relatively high concentration of Cd may be an effect of industrial pollution, as well as other activities of anthropogenic origin (Srebocan et al. 2006). Due to the ability to accumulate in living organisms, Cd is a marker of environmental pollution (Ikeda et al. 1996). In contrast to Cu and Zn and other necessary micro- and macroelements, Cd accumulates in mammalian tissues proportionally to its concentration in the environment.

Cd is one of the cumulative toxin. It is absorbed, quickly passes from the blood to various organs, mainly concentrates in the liver and kidneys, where it is bound to metallothioneins. Metallothioneins are low-molecular proteins containing numerous cysteine residues involved in the detoxification of organisms from harmful metal ions and the oxidative stress defense mechanisms. High concentrations of Cd may cause negative consequences for the body, i.e., damage of the kidney tubules, degeneration of the testis and ovaries (Toman et al. 2002).

In conclusion, the liver concentrations of Se, Zn, Cu, Cd in selected species of wild animals: the red fox, wild boar and red deer from the hunting areas of Warmia and Mazury, do not differ from the standard values. These results do not diverge from the literature data. A low concentration of Se may be related to the low level of this element in the studied area. The Cd and Zn levels correspond with the low level of these heavy metals in other parts of Poland.

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