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Michał SKIBNIEWSKI, Ewa M. SKIBNIEWSKA[1], Aneta GAŁĄZKA[1], Marta KOŁNIERZAK[1], Hubert KMIEĆ[1]

THE CONTENT OF Cd, Pb, Cu, Zn AND Mo IN THE SPLEEN OF MOOSE (*Alces Alces* L.) FROM NORTHEASTERN POLAND

ZAWARTOŚĆ Cd, Pb, Cu, Zn I Mo W ŚLEDZIONIE ŁOSI (*Alces Alces* L.) Z PÓŁNOCNO-WSCHODNIEJ POLSKI

Department of Morphological Sciences, Warsaw University of Life Sciences – SGGW, Poland
[1]Department of Biology Environment Animals, Warsaw University of Life Sciences – SGGW, Poland

Streszczenie. Celem badań była ocena zawartości metali ciężkich: kadmu (Cd), ołowiu (Pb) oraz mikroelementów: miedzi (Cu), cynku (Zn) i molibdenu (Mo) w śledzionie łosi pochodzących z północno-wschodniej Polski. Zwierzęta zostały podzielone na dwie grupy wiekowe (jedna obejmowała osobniki młodociane w wieku do dwóch lat, druga – zwierzęta powyżej dwóch lat). Zawartość pierwiastków w zebranych próbkach oznaczono za pomocą metody spektrometrii mas z plazmą wzbudzoną indukcyjnie (ICP-MS). Średnia zawartość metali w badanych narządach wynosiła odpowiednio: 2,28, 0,10, 8,36, 25,29 i 0,22 mg · kg^{-1} świeżej masy. Większe wartości odnotowano u osobników starszych, jednak nie stwierdzono istotnych różnic statycznych między grupami. Analizując relacje między wybranymi pierwiastkami, stwierdzono istotne statystycznie korelacje między cynkiem a molibdenem (r = 0,58), kadmem a miedzią (r = 0,56) oraz kadmem a molibdenem (r = 0,55). Zawartość kadmu, ołowiu, miedzi, cynku i molibdenu w śledzionie badanych łosi była większa u osobników starszych. Pomimo że nie ma wartości referencyjnych dotyczących zawartości kadmu i ołowiu w śledzionie łosi, odnotowane wartości należy traktować jako duże, ponieważ odpowiadają one wartościom rejestrowanym u innych gatunków przeżuwaczy bytujących w rejonach uprzemysłowionych. Zjawisko to wynika z obecności wśród badanych zwierząt niewielkiej liczby starszych osobników, u których odnotowano duże zawartości wymienionych metali.

Key words: moose (*Alces alces*), spleen, heavy metal, trace element.
Słowa kluczowe: łosie (*Alces alces*), śledziona, metale ciężkie, mikroelementy.

INTRODUCTION

Free-living animals play an important role in the assessment of environmental contamination with heavy metals. Moose is a representative of the biggest free-living ruminants of the northern hemisphere. Results of the performed investigations show that the analysis of the content of heavy and biogenic metals in the tissues of that animal comprises a collection of valuable information on the state of the environment of its living conditions (Custer et al. 2004; Arnold et al. 2006; Danielsson and Frank 2009; Skibniewski et al. 2017).

Corresponding author – Adres do korespondencji: Ewa Skibniewska, Department of Biology Environment Animals, Warsaw University of Life Sciences – SGGW, Ciszewskiego 8, 02-786 Warszawa, Poland, e-mail: ewa_skibniewska@sggw.pl

In Poland this species is under a specific type of protection. Although it is counted among the game animals, however, since 2001 there is a moratorium on the shooting of moose set out by the Ministry of Environment. Its introduction allowed the restoration of moose population in Poland which in the sixties and seventies of the 20th century was on the border of extinction. At present the size of moose population is estimated at about 7.5 thousand of animals, most of which live in the Northeastern regions of Poland that is Podlasie, Warmia and Mazury and Mazovia provinces (Budny et al. 2010; Central Statistical Office 2015). Although the number of moose in Poland grows, what may suggest their good living condition, however, there is a limited knowledge about the real state of their health. Especially interesting is the state of mineral supply as well as the content of heavy metals in their tissues and organs. Adult animals of that species practically have no natural enemies which enables them to have a relatively long life.

Thus the analysis of the heavy metal content in particular organs of their organisms seems to be a valuable element of the environmental monitoring in relation to their long- lasting exposition. It is important due to the risk of the possible contaminations with toxic metals like cadmium and lead, which are commonly present in the environment. Their concentration in the organs reflects not only the environmental bioavailability but also depends on the species, age, gender and physiological state of the animal. Due to great mobility of cadmium in the environment and extreme susceptibility to bioaccumulation it is gradually stored in the trophic chain. (Kabata-Pendias 2000; Kośla et al. 2008; Danielsson and Frank 2009; Skibniewski et al. 2017). Lead belongs to the most toxic heavy metals. For a long time it was classified into elements with embryotoxic, teratogenic, mutagenic and carcinogenic properties. Both elements get into the organism mainly through the alimentary tract and through the respiratory system (Jakubowski et al. 1997; Krzywy et al. 2010; Curi et al. 2012). Apart from toxic metals, the knowledge about essential elements such as copper, zinc and molybdenum is also important since they are included in microelements taking part in many biochemical reactions conditioning the maintaining of homeostasis. In ruminants copper and molybdenum are the metals of special importance due to their strong antagonism. It has been shown that molybdenum poisoning may appear even with the proper copper tissue content (Frank et al. 2000; Prasad 2002; Kośla et al. 2004; Rink and Haase 2007; Zatta and Frank 2007; Tubek et al. 2008; Schwarz et al. 2009).

The performed investigations aimed at analysing the content of some chosen metals in the spleen of moose. They will be used for creating a database concerning the content of elements in those species of animals whose mineral status in Poland as opposed to other regions of the world is completely unknown. The supplemented knowledge on the state of mineral supply of moose together with the further investigations carried out by other research teams and concerning, among others, genetic analysis, moose reproduction and field observations regarding the migration of those animals will allow a complex study of the biology of this species.

MATERIAL AND METHODS

The experimental material comprised samples of spleens (of about 5 g each) collected from 14 female moose from Northeastern Poland, which is an unpolluted region with

numerous protected areas being refuges of moose and other representatives of deer family (Fig. 1). Samples were obtained during selective shots in 2010 on the basis of the permission of the Minister of Environmental Protection (decision number DL.gł-6713-5/45392/10/PJ).

Fig.1. Geographical location of the sampling sites
Ryc. 1. Miejsca pobierania prób

Animals were divided into two groups depending on their age (animals up to 2 years of age [n = 7] and over 2 years [n = 7]). Division into groups was established on the base of animals puberty time, which in moose females takes place about the end of the 2nd year of their life. The first group comprised of juvenile animals whereas the second consisted of fully mature individuals. Mean age of animals was 6 years. None of the animal showed the symptoms of splenomegaly. The collected tissue samples was then placed in plastic containers and stored at −20°C. Directly prior to the determination of the metal content, samples were homogenized and then placed in high-pressure teflon containers. Mineralization was carried out in a Milestone microwave system. The validation of that method was performed in relation to the certified reference material CRM–BCR 185 R (Community Bureau of Reference, BCR in Brussels, Belgium). The concentration of elements in the investigated samples was determined by the inductively coupled plasma mass spectrometry (ICP-MS). All results are presented in the average form of the measurements expressed in mg · kg^{-1} fresh weight of the investigated organs.

Statistical analysis of the data was performed using the Statistica 12™ software (StatSoft, Inc.). Prior to the analysis the data were examined in order to determine their distribution using the test W Shapiro-Wilk. The concentrations of elements did not get the normal distribution thus in order to test the significance of differences between the investigated groups the non-parametric methods were used. The significance of differences (at $p \leq 0.05$, $p \leq 0.01$) was assessed using the U Mann-Whitney test. Analysis of correlations between the given parameters was performed using the Speraman's correlation.

RESULTS AND DISCUSSION

Spleens of various animal species are characterized by certain morphological differences. In mammals two basic functional types of that organ are recognized. They are conventionally described as storage spleen and defensive spleen. Storage spleen is considered to be an organ modifying the composition and volume of circulating blood. Species with that type of spleen are, among others, dogs, cats, cattle and non domestic ruminants. Organ of that type is characterized by the fact that during rest it contains significant amounts of erythrocytes and is relatively large as compared to the animal body size. Due to its volume, during the contraction of the capsule, spleen of that type can exert a significant influence on the concentration of erythrocytes in circulating blood (Bowdler 2002). In the accessible literature there are no reports concerning the content of heavy and biogenic metals in the moose spleen. Because of that, the results obtained can be compared only with the data concerning other free-living species and domestic ruminants. With the exception of two works by Skibniewski et al. (2016, 2017) the content of heavy and biogenic metals in the parenchymatous organs of moose from Poland has not been studied.

Cd, Pb. The concentration of elements in the investigated organs are presented in Table 1. The main organs storing up cadmium are kidneys in which the process of its deposition is closely correlated with the animal age. The data concerning the cadmium content in the spleen of ruminants are fragmentary and concern mainly the domestic animals. In animals of that group of herbivores the value accepted as normal amounts to 0.31 mg · kg^{-1} of the organ dry matter while the increased cadmium concentrations begin above 1.28 mg · kg^{-1} dry matter of the organ. After including the degree of the spleen hydration amounting to 76%, these values amounted to: 0.074 and 0.31 mg · kg^{-1} fresh matter (Puls 1994). Kar et al. (2015) observed that in goats cadmium concentrations amounted on the average to 0.06, 0.36, 0.57 and 1.17 mg · kg^{-1} fresh weight in juvenile and mature animals from reference and polluted areas respectively. Rogowska et al. (2008) noted that the average content of cadmium in the spleen of sheep from Poland amounted to 0.195 mg · kg^{-1} wet weight.

Table 1. The analysed metal concentrations in the spleen of moose [mg · $kg^{-1}$of wet weight]
Tabela 1. Zawartość badanych metali w śledzionach łosi [mg · $kg^{-1}$of świeżej masy]

Metals Metale	All animals Wszystkie zwierzęta (*n* = 14)				Animals up to 2. years Zwierzęta w wieku do 2 lat (*n* = 7)	Animals older than 2. years Zwierzęta powyżej 2 lat (*n* = 7)
	arithmetic mean średnia arytmetyczna	standard deviation odchylenie standardowe *SD*	range zakres	median mediana	mean ± *SD* średnia ± *SD*	
Cd	2.28	7.63	0.04–28.79	0.09	0.05 ± 0.02	4.52 ± 10.71
Pb	0.10	0.17	0.04–0.72	0.05	0.05 ± 0.03	0.15 ± 0.25
Cu	8.36	16.17	0.54–52.30	0.95	1.14 ± 0.05	15.56 ± 21.08
Zn	25.29	8.23	14.00–43.00	24.50	20.86 ± 4.22	29.71 ± 9.12
Mo	0.22	0.31	0.04–0.88	0.04	0.04 ±0.03	0.40 ± 0.36

The mean values obtained in our own investigations is over seven fold higher than the data by Puls (1994) concerning the increased cadmium content in the cattle spleen. It results from the presence in the group of the investigated animals one animal with an extreme high level of that metal in the spleen amounting to 28.79 mg · kg^{-1} fresh weight. The value of the median in the investigated group is similar to the data concerning cattle and amounts to 0.085 mg · kg^{-1} fresh weight of the spleen.

Lead is a heavy metal which increased concentration in the tissues of game animals mainly results from the contamination from the used jacket bullets which contain that metal. In Poland the cases of lead contamination of the tissues of game animals have been observed for many years (Jarzyńska and Falandysz 2011). For the first time attention was drawn to that fact by Falandysz and Caboń (1990). It mainly concerns the muscle tissue which is the valuable component of a human diet. Thus the area of a gunshot wound should be carefully removed together with a proper margin of the tissue thus guaranteeing an effective removal of the remains of the lead bullet (Jarzyńska and Falandysz 2011). Similarly as in the case of cadmium, there are no reports in the accessible literature concerning the lead content in the moose spleen. The investigations performed so far are mainly concerned with its content in the kidneys and liver (Puls 1994).

While investigating the lead content in the spleen of various species of ruminants from the contaminated areas of Egypt, Abou Donia (2008) observed that in buffalo it amounted on the average to 0.01 to 0.056 mg · kg^{-1} fresh weight depending on the area of the animal living. Higher values were observed in animals from the industrial areas. The lead content in the spleen of cattle amounted to from 0.013 to 0.046, in sheep from 0.098 to 0.110, while in the case of goats from 0.006 to 0.052 mg · kg^{-1} fresh weight. In the representatives of deer family living in the analysed regions the lead content in the spleen was from 0.010 to 0.048 mg · kg^{-1} fresh weight. Stavreva-Veselinovska and Živanovič (2010) observed that the average content of lead in the spleen of goats and sheep from industrial region is: 0.115 and 0.031 mg · kg^{-1} fresh weight respectively. Hassan et al. (2013) reported that the concentrations of lead in the examined samples of spleen of cows ranged from 0.12 to 1.08 with a mean value of 0.57 mg · kg^{-1} wet weight.

The results obtained in our own investigations in which the median of the lead content in the spleen of moose amounted to 0.045 mg · kg^{-1} fresh weight are close to the highest values noted in the spleen of buffalos, cattle, goats and cervids. Due to the above data the lead content in the spleen of Polish moose should be accepted as high because it corresponds to the values registered in the organs of animals living in the highly industrial regions. A relatively high lead content in the spleen of the investigated moose obtained from the regions of a relatively low degree of industrialization cannot be connected with the presence of the bullet remains because none of the analysed organs had the signs of the gunshot wounds.

Zn, Cu, Mo. Similarly as in heavy metals also in the case of biogenic elements there are no reports in the accessible literature concerning their content in the spleen of moose. The obtained results of the investigations mainly concerns the liver and kidneys (Parker and Hamr 2001; Custer et al. 2004; Frank et al. 2004; Skibniewski et al. 2017). While analyzing the zinc content in the kidneys and liver of moose originating from Ontario province Parker

and Hamr (2001) observed that the average content of that metal in the kidneys of calves amounted to 173.41 mg · kg^{-1} dry matter while in adult animals to 164.47 mg · kg^{-1}. In the samples of liver these values amounted to 73.62 and 74.77 mg · kg^{-1} dry matter, respectively. After the conversion of the obtained results into their content in fresh weight they amounted to 34.7, 32.9, respectively in the kidneys and 21.42 and 21.75 in the liver. Frank et al. (2004) observed that the median of zinc content in the kidneys and liver of moose from New Scotland amounted to 50 and 46 mg · kg^{-1}, respectively. Custer et al. (2004) noted that the average content of zinc in the kidneys of dead moose found in the region of north-western Minnesota amounted to 33.4 mg · kg^{-1} dry matter in animals living on the agricultural lands and prairie and in animals living in peatbogs and forests to 43.8 mg · kg^{-1}. An average zinc content in the kidneys of animals from the regions of Yukon amounted to 29.24 mg · kg^{-1}, while in the liver to 34.87 mg · kg^{-1} fresh weight (Gamberg et al. 2005). In moose from the region of Sweden the content of zinc in the liver of healthy animals amounted to 54.8 mg · kg^{-1} fresh weight while in animals showing the symptoms of the, so called, mysterious disease of moose – 66.8 mg · kg^{-1}. In the kidneys of healthy animals the content of zinc amounted to 40.6 mg · kg^{-1}, on the average, while in diseased animals to 48.5 mg · kg^{-1} (Frank et al. 2000). The content of zinc in the kidneys of healthy animals was close to the average. In 422 animals from Norway the content of zinc in the liver amounted, on the average, to 31.4 mg · kg^{-1} fresh weight (Vikøren et al. 2011). In relation to domestic cattle, in animals suffering from zinc deficiency its content in the parenchymal organs dropped below 20 mg · kg^{-1} fresh weight (Puls 1994). In the kidneys of healthy animals the content of zinc amounted, on the average, to 40.6 mg · kg^{-1}. The zinc level found as optimal in the liver amounted to from 25 to 100 mg · kg^{-1} while in the kidneys from 18 to 25 mg · kg^{-1} fresh weight of the investigated tissue.

Due to the lack of data in the accessible literature which makes it impossible to carry out a direct comparison it is not possible to explicitly interpret the obtained results. However, their values are close to the proper zinc level in the kidneys of cattle and moose living in the different regions of the world.

An important problem is an interaction between zinc and cadmium taking place in the organism. Both metals have the ability to induce the process of producing metallothioneins in which, at the same time, they compete for the thiol group of the metallothionein (Martelli et al 2006). At first it was thought that the only function of metallothionein is the protection against the toxic action of heavy metals (mainly cadmium) because it was isolated for the first time in 1957 from the renal cortex of a horse as a protein bonding cadmium (Thirumoorthy et al. 2011). At present it is known that proteins of that group act as regulators of zinc and copper metabolism and protect cells against damages caused by the alkylating agents, free radicals and ionizing radiation.

The authors of the present work have not found any investigations on the dependence between the concentration of cadmium and zinc in the spleen of moose. In the analysis of the interaction between the mentioned metals no statistically significant dependencies were observed. The correlations between the metal concentrations in particular organs are presented in Table 2. A significantly correlated dependence was observed between the cadmium content and both metals (copper and molybdenum) in the investigated organs. The highest positive correlation ($r = 0.58$, $p \leq 0.05$) was noted between the molybdenum and zinc.

Table 2. The correlation coefficients between metal contents in the spleen of moose
Tabela 2. Korelacje między zawartościami metali w śledzionach łosi

Elements Pierwiastki	Cadmium Kadm	Lead Ołów	Copper Miedź	Zinc Cynk
Lead Ołów	0.52			
Copper Miedź	0.56*	0.12		
Zinc Cynk	0.48	0.28	0.33	
Molybdenum Molybden	0.55*	0.34	0.43	0.58*

* correlation coefficient significant at $p \leq 0.05$ – zależności istotne przy $p \leq 0,05$.

In the accessible literature some data could be found concerning the copper content in the spleen of ruminants. Puls (1994) noted that the proper concentration of that metal in the spleen of domestic cattle was included within the limits from 4 to 6 mg · kg^{-1} dry matter of the organ. After taking into consideration the degree of hydration of the spleen those values amounted to 0.96 and 1.44 mg · kg^{-1} fresh weight, respectively. Kar et al. (2015) found that mean copper concentration in the spleen of goats older than 1 year from unpolluted region amounted to 0.58 mg · kg^{-1}, while in animals from polluted regions it amounted to 3.07 mg · kg^{-1} wet weight. Results obtained in our own investigations are similar to the lower limit of the reference values concerning cattle. The median of copper content in the spleen of the investigated moose amounted to 0.95 mg · kg^{-1}. It should be stressed that the obtained results were characterized by significant differences between certain individuals. In the analysis of interactions between the investigated elements there was observed the presence of a statistically significant ($p \leq 0.05$) positive correlation between the cadmium and copper contents.

Copper and molybdenum play an important role in ruminants because of a certain competitiveness of both metals in metabolic processes. An excess of molybdenum in a diet causes a secondary copper deficiency because thiomolybdates appear in the rumen in the presence of the sulphates (Dziekan et al. 2007; Gould and Kendall 2011). A particular significance of both metals appeared in moose from the region of Sweden in which, as it was already mentioned, in the middle of the 80's of the 20th century, the mysterious disease of moose appeared which resulted from molybdenosis causing a secondary copper deficiency (Frank et al. 2002, 2004). The results of the published investigations consider mainly the contents of the mentioned metals in the liver which is the main organ storing up copper. Up till now no data were published concerning the spleen. While investigating animals from the region of Sweden, Frank et al. (2000) observed that the average content of copper in the liver of clinically healthy animals comprising the control group amounted to 29.0 mg · kg^{-1} wet weight and in diseased animals it amounted to 11.4 mg · kg^{-1}, respectively. No presence of statistically significant differences were observed between the investigated groups in which the copper content was within the quite wide limits. In clinically healthy animals those values amounted to from 3.93 to 106 mg · kg^{-1}, while in diseased animals from 3.2 to 28.2 mg · kg^{-1}. Results obtained in the authors' own investigations are close to those registered in healthy animals from Sweden. The average content of copper amounted to 23.07 mg · kg^{-1} and the values varied from 1.16 to 87.42 mg · kg^{-1}. The molybdenum content in the liver of healthy

animals from Sweden amounted to, on the average, 0.855 mg · kg^{-1}, while in the livers of diseased animas from that region it was 1.165 mg · kg^{-1}. Both investigated groups differed in a statistically significant way (Frank et al. 2000). The copper content in the kidneys of healthy animals from Sweden amounted on the average to 3.8 mg · kg^{-1} fresh weight of the investigated tissue while that value in diseased animals was 5.469 mg · kg^{-1}. In other investigations which were published by Frank et al. (2004) concerning the content of copper and molybdenum in the liver of salmons from Sweden the median for copper amounted to 34 mg · kg^{-1} fresh weight while in the case of molybdenum 0.82 mg · kg^{-1}.

RECAPITULATION

Analysis of the heavy metal content in studied organs of moose seems to be a valuable element of the environmental monitoring. Based on the analysis of cadmium, lead, copper, zinc, and molybdenum levels in the moose spleen, it was found that the mean concentration of the analysed metals was higher in the group of the older individuals. The mean cadmium and lead concentrations in the animals studied can be considered high because they correspond to the values registered in the organs of other ruminant species living in the industrial regions. Although animals studied originate from relatively unpolluted areas we noted the presence of older individuals with high levels of those metals in the spleen.

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Abstract. The study was performed to assess the concentrations of heavy metals: cadmium (Cd) and lead (Pb) as well as essential elements: copper (Cu), zinc (Zn) and molybdenum (Mo) in the spleen of moose from Northeastern Poland. The animals studied were divided into two age groups (juvenile individuals up to 2 years old and mature animals older than 2 years).The concentrations of selected elements in the collected samples was determined by the inductively coupled plasma mass spectrometry (ICP-MS). The mean concentrations in the organs examined were: 2.28, 0.10, 8.36, 25.29 and 0.22 mg · kg^{-1} wet weight, respectively for cadmium, lead, copper, zinc and molybdenum. Although higher values were found in older individuals, both age groups did not differ statistically. In the analysis of relations between selected metals we noted statistically significant correlations between following pairs of elements: zinc and molybdenum (r = 0.58), cadmium and copper (r = 0.56) and cadmium and molybdenum (r = 0.55). The cadmium, lead, copper, zinc, and molybdenum levels in the moose spleen was higher in the group of the older individuals than in the younger ones. Although there are no reference values for the cadmium and lead concentrations in the moose spleen the mean values observed can be considered high because they correspond to the levels registered in the organs of other ruminant species living in the industrial regions. This phenomenon is due to the presence of a few older individuals with high levels of those metals in the spleen among investigated animals.

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