e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 66 (2018), No 3
https://doi.org/10.3409/fb_66-3.14

The Content of Mineral Elements and Heavy Metals in the Hair of Red Deer (*Cervus elaphus* L.) from Selected Regions of Poland

Dorota CYGAN-SZCZEGIELNIAK✉, Magdalena STANEK, Karolina STASIAK, Aleksandra ROŚLEWSKA, and Bogdan JANICKI

Accepted November 29, 2018 *Published online November 30, 2018* *Issue online November 30, 2018*

Original article

CYGAN-SZCZEGIELNIAK D., STANEK M., STASIAK K., ROŚLEWSKA A., JANICKI B. 2018. The content of mineral elements and heavy metals in the hair of red deer (*Cervus elaphus* L.) from selected regions of Poland. Folia Biologica (Kraków) **66**: 133-142.

The aim of the research was to determine the influence of geographical location on the content of mineral elements (Zn, Cu, Mn, Fe, Ca, Mg, Na, K) and heavy metals (Pb and Cd) in the hair of red deer (*Cervus elaphus* L.). The experimental material comprised samples of hair collected from 2-3-year-old male red deer from three Polish provinces: Kuyavian-Pomeranian (n=15), Lesser Poland (n=15) and Masovian (n=15). The analysis of content of Zn, Cu, Mn, Fe, Ca and Mg was conducted by means of atomic absorption spectroscopy (AAS), and in the case of Na and K – of atomic emission spectroscopy (AES). The content of Pb and Cd was determined in a certified laboratory, using electrothermal atomic absorption spectroscopy (ASS-ET). The highest concentration of Pb was noted in the samples from the Masovian Province and Cd – from the Lesser Poland Province. The differences between the regions in terms of the content of quantity elements and essential trace elements were statistically significant and showed much variety (with the exception of Cu). The analysis of correlation revealed positive and statistically important interactions between numerous elements, among which the most significant were observed in the pairs Na-K (r_{xy}=0.77; p<0.01) and Cu-Mn (r_{xy}=0.82; p<0.01). Only one negative and high statistically important relationship was noted – in the case of Cu and Zn (r_{xy}= -0.71; p<0.01).

Key words: Quantity elements, essential trace elements, heavy metals, hair, red deer.

Dorota CYGAN-SZCZEGIELNIAK✉, Magdalena STANEK, Karolina STASIAK, Aleksandra ROŚLEWSKA, Bogdan JANICKI, Department of Physiology, Animal Physiotherapy and Animal Breeding, UTP University of Science and Technology, Mazowiecka 28, 85-064 Bydgoszcz, Poland.
E-mail: cygan@utp.edu.pl

Heavy metals and other toxic substances present in the natural environment can be a source of grave health problems, as they strongly influence the organisms. Not only can exposure to high doses be hazardous, but low concentrations may also pose a threat if they act permanently. Heavy metals accumulate mainly in bones, hair, internal organs, tissues and muscles, as a result of their penetration via three main routes, i.e. respiratory, topical or enteral, by their uptake with water or food (MICHALAK *et al.* 2012; WONGSASULUK *et al.* 2018). One of the indicators which may serve as a basis for the biological identification of trace elements in the environment can be the aforementioned hair or fur (JAUHARAH *et al*. 2013). Moreover, the assessment of the levels of mineral elements and heavy metals in animals' coats can be regarded as a perfect source of information, both about their exposure at their place of living, and on their state of health and nutrition. Heavy metals are among the most dangerous contaminants in the environment, due to their toxicity and their potential for accumulation, which often surpasses the organism's capability of detoxification (JAUHARAH *et al.* 2013). The elemental analysis of hair or fur allows the assessment of the long-term exposure of the organisms to those substances, which makes it a more reliable method of monitoring their state and health than blood or urine tests, the results of which are often affected by the temporary influence of some factor. It is a widely known fact that there is a close relationship between the elements

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determined in hair and their concentration in the body (CHO 1997).

Wild animals, due to their close connection with the particular place they inhabit, are highly susceptible to the effects of various environmental factors. The deficiency of quantity elements or the accumulation of hazardous heavy metals in tissues can significantly affect their health, and in extreme situations result in the intoxication of individuals and the shrinking of populations. Only preserving the optimum ratio between the elements at all levels of metabolic processes allows the maintenance of the organism's homeostasis (FRENCH *et al.* 2017). Red deer is a game species. Due to its full integration with the environment throughout its whole lifespan, it is a good marker of environmental pollution as well as an experimental model. The animals usually stay within the same specified region, which allows the determination of the level of contamination of the given location. Due to the accumulation of various mineral compounds and elements in their soft tissue, bones and hair, a mineral profile of their bodies can be obtained.

Obtaining samples of animal hair is simple and non-invasive, and thus it is a widely applied method in studies involving biological monitoring. (DUNNETT & LEES 2003; GRATACÓS-CUBARSÍ *et al.* 2006). The hair of wildlife and farm animals can be an ideal bioindicator of nutrition mistakes being a consequence of inadequate meeting of the mineral requirements of animals, as well as soil, water and air pollution (CYGAN-SZCZEGIELNIAK *et al.* 2014; PATKOWSKA-SOKOŁA *et al.* 2009; SZKODA & ŻMUDZKI 2001; TYMCZYNA *et al.* 2000).

The aim of the study was to determine the influence of geographical location on the content of mineral elements (Zn, Cu, Mn, Fe, Ca, Mg, Na, K) and heavy metals (Pb and Cd) in the hair of red deer (*Cervus elaphus* L.) from three Polish provinces: Kuyavian-Pomeranian, Lesser Poland and Masovian.

Material and Methods

The experimental material comprised samples of hair collected from 2-3-year-old male red deer from three Polish provinces: Kuyavian-Pomeranian (n=15), Lesser Poland (n=15) and Masovian (n=15). The animals were shot during the hunting season of 2014 by individual hunters on shooting grounds within the selected regions in accordance with the DECREE OF THE MINISTRY OF ENVIRONMENT from 16 March 2005 (Journal of Laws No 48, item 459). The samples were collected between August 21 and October 30, and therefore they represent the so-called "summer hair".

The Kuyavian-Pomeranian Province is a moderately industrialized region. The main sectors active within its area are the chemical, electrical and machine industries, food processing, the cellulose industry and printing services. The air analysis performed in the years 2013-2015 showed a downtrend in the concentration of SO_2, NO_2 and dust in this region (THE REPORT on the state of the environment Kuyavian-Pomeranian Province in the years 2013-2015).

The quality of the air in the Masovian Province is a resultant of local anthropogenic emissions, as well as, to a considerable extent, the inflow of pollutants from other parts of Poland and Europe. The main local sources of contamination are individually heated houses, and areas with roads of high traffic density. According to the data presented by the Central Statistical Office (CSO), in 2014 this province was the third biggest source of gas and dust pollution in Poland (STATE OF THE ENVIRONMENT in the Masovian Province in 2015).

In the Lesser Poland Province the leading sources of air pollution are: anthropogenic emissions stemming chiefly from industrial activity (stationary sources) and traffic (mobile sources). Industrial emissions are organized and mostly related to burning fuels (electric plants, heating plants, electric and heating plants) and various kinds of technological processes (industrial establishments) (THE REPORT on the state of the environment Lesser Poland Province in the years 2013-2015).

The samples of hair were collected from the animals' sides, immediately behind the arcus costalis, using specialist laboratory stainless steel scissors, which allowed us to obtain the samples in any environmental conditions without the risk of their chemical contamination. The samples were cut just over the skin, from an area of 10x10 cm. Until further analysis they were stored in a tightly closed polythene bag, in a dry and shaded place. In order to remove the dirt and fat the samples were washed in acetone and placed for 15 minutes in an ultrasonic cleaner. They were stored afterwards for 12 hours. After removing the acetone (by means of decantation), the hair was rinsed twice with distilled water and dried in a drying oven at a temperature below 50°C. An adequate procedure of preparing the hair for analysis ensured that it was free of any potential chemical contamination which may have been present on its surface.

The prepared hair was wet mineralized using Ethos Plus (Milestone) microwave mineralizer, conformingly to the POLISH STANDARD PN-EN 13805: 2014. For this purpose, an aliquot of 0.20 g was prepared, which was subsequently treated with 6.25 cm^3 of HNO_3 (65%) and H_2O_2 (30%) mixture, in a 4:1 (v:v) volume ratio. The process of mineralization lasted 20 minutes. During the first 10 minutes the temperature rose to 190°C, and it was afterwards sustained at a level of 190°C±5°C. After mineralization the samples were transferred quantitatively to 25 ml volumetric flasks and the volume was adjusted with distilled water. The content of mineral elements in hair was expressed in g/kg and mg/kg of dry matter.

The analysis of the content of zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), magnesium (Mg) and calcium (Ca) was performed by means of atomic absorption spectroscopy (AAS), while the content of Na and K was measured with atomic emission spectroscopy (AES). The concentration of lead (Pd) and cadmium (Cd) was determined using electrothermal atomic absorption spectroscopy (ASS-ET). The measurements were performed in a certified laboratory, in accordance with methods by CHATT and KATZ (1989) and conforming to the POLISH STANDARD PN-EN-14084: 2004.

Statistical analysis

Some results did not meet the assumptions of normal distribution (which was verified using the Shapiro-Wilk test) or the assumptions of homoscedasticity, required for implementing parametric tests, so in order to examine the statistically significant differences between the experimental groups the non-parametric Kruskal-Wallis test (non-parametric ANOVA) was used. For the parameters meeting the assumptions of normal distribution and homogeneity of variance, the parametric analysis of variance (ANOVA) and afterwards a test for multiple comparisons, i.e. post-hoc Tukey test, ware applied. Due to the fact that the difference between the mean values recorded for the groups (locations) did not affect significantly the trend / relation between the elements (which was investigated for each location), those interrelations were presented in the paper in one collective table, instead of dividing them into groups (locations). For the purpose of analyzing the relations between all elements in question regardless of the location from where the samples were obtained Spearman's rank correlation coefficient was applied. Moreover, for the elements which did not meet the assumptions of normal distribution, median (Me) was calculated. The obtained results were processed statistically using Statistica 12.0 software.

Results

The tables present the content of heavy metals (Table 1), quantity elements (Table 2) and essential trace elements (Table 3) in the examined material, as revealed by the experiment. The content of heavy metals, i.e. cadmium and lead, in the hair of deer showed statistically significant differentiation depending on the location of the samples' collection, i.e. the selected provinces. In the case of lead, the highest values were recorded for Lesser Poland and Masovian provinces, while in the hair of the animals from the Kuyavian-Pomeranian Province this concentration was lower by 30%. In general, the content of this element in the matrix under investigation, without considering the location of the animals' habitat, ranged from 7.54 to 10.16 mg/kg. As regards cadmium, the lowest concentrations were obtained from the hair of deer from Kuyavian-Pomeranian and Masovian provinces, and were higher by 35% in the case of Lesser Poland (Table 1). The amount of this heavy metal, without analyzing the place of origin of the sample, ranged from 0.12 to 0.18 mg/kg. All quantity elements in the hair of red deer under investigation showed significant differentiation depending on the location/province. On the basis of the results obtained, no area can be indicated beyond question as associated with highest or lowest amounts of the majority of the elements under the study (Table 2). This is not the case with essential trace elements. For Zn and Fe higher values were recorded in the hair of deer from Lesser Poland. The highest concentration of Mn was marked in the hair of animals from Kuyavian-Pomeranian and Lesser Poland provinces, while in the hair of deer from the Masovian Province this value was lower by approximately 28%. It is also noteworthy that an almost identical concentration of Cu was found in the hair obtained from all the three provinces, and its value in all cases remained within the level of 18 mg/kg (Table 3). Taking into account the interactions between the elements, the following two positive correlations of high statistical significance were noted: between Mn and Cu (r_{xy}=0.82; $p<0.01$), as well as between K and Na (r_{xy}=0.77; $p<0.01$). A negative correlation of high statistical significance was also observed between Cu and Zn (r_{xy}=-0.71; $p<0.01$). The remaining positive and statistically important correlations are presented in Table 4.

Discussion

Wild deers' diet consists mostly of plants, fresh water, as well as minerals from the soil, which may penetrate into the animal's body with consumed roots or during licking. It may also contain anthropogenic contamination, as the soil, especially at the roadside, is likely to include considerable amounts of toxic heavy metals (FRENCH *et al.* 2017). Thus, the animals' diet and environmental pollution have a grave impact on the concentration

Table 1

The content of lead and cadmium in the hair of red deer from the selected provinces (mg/kg) dry matter

Element	Kuyavian-Pomeranian (n=15)	Lesser Poland (n=15)	Masovian (n=15)
	$\bar{x}\pm SD$	$\bar{x}\pm SD$	$\bar{x}\pm SD$
*Pb	$7.54^a\pm0.60$	$9.46^b\pm0.94$	$10.16^b\pm0.70$
*Cd	$0.12^a\pm0.03$	$0.18^b\pm0.01$	$0.12^a\pm0.02$

[a, b, c] The mean values marked with different letters vary significantly ($p<0.05$); n – number of samples/animals; SD – standard deviation. The example of interpreting the marking: [a] differs from [b] in the same row at $p<0.05$

*For Pb and Cd the differences between the groups were analyzed using a parametric test (see the methodology).

Table 2

The content of the selected quantity elements in the hair of red deer from the compared provinces (g/kg) dry matter

Element	Kuyavian-Pomeranian (n=15)		Lesser Poland (n=15)		Masovian (n=15)	
	$\bar{x}\pm SD$	Me	$\bar{x}\pm SD$	Me	$\bar{x}\pm SD$	Me
*Ca	$10.98^a\pm0.97$	10.39	$9.37^b\pm1.41$	8.96	$12.07^c\pm0.41$	11.04
*Mg	$0.68^a\pm0.41$	0.62	$0.67^a\pm0.19$	0.60	$0.58^b\pm0.51$	0.46
*Na	$3.43^a\pm0.75$	3.60	$4.13^b\pm0.81$	4.06	$3.15^a\pm0.93$	2.81
*K	$1.11^a\pm0.39$	0.96	$1.43^b\pm0.45$	1.48	$1.80^c\pm0.49$	1.54

[a, b, c] The mean values marked with different letters in the same row vary significantly ($p<0.05$); Me – median (Q_2); n – number of samples/animals; SD – standard deviation

*For Ca, Mg, Na and K the differences between the groups were analyzed using a nonparametric test (accordingly to the methodology).

Table 3

The content of zinc, copper, manganese (mg/kg) dry matter and iron (g/kg) dry matter in the hair of red deer from the selected provinces

Element	Kuyavian-Pomeranian (n=15)		Lesser Poland (n=15)		Masovian (n=15)	
	$\bar{x}\pm SD$	Me	$\bar{x}\pm SD$	Me	$\bar{x}\pm SD$	Me
*Zn	$142.63^a\pm7.66$	147.85	$158.28^b\pm4.24$	147.30	$114.98^c\pm9.71$	105.20
*Cu	$17.98^a\pm2.96$	16.95	$17.52^a\pm2.25$	17.65	$17.96^a\pm2.75$	19.15
*Mn	$19.29^a\pm6.71$	20.31	$18.40^a\pm1.22$	16.20	$13.87^b\pm5.50$	13.80
*Fe	$0.25^a\pm0.15$	0.26	$0.33^b\pm0.31$	0.25	$0.30^b\pm0.19$	0.31

[a, b, c] The mean values marked with different letters in the same row vary significantly ($p<0.05$); Me – median (Q_2); n – number of samples/animals; SD – standard deviation

*For Zn, Cu, Mn and Fe the differences between the groups were analyzed using a nonparametric test (accordingly to the methodology)

Table 4

Spearman's correlation coefficients (r_{xy}) between the elements present in hair of red deer

Variable	Pb	Cd	Ca	Mg	Na	K	Zn	Cu	Fe
Cd	0.22								
Ca	-0.20	0.29							
Mg	-0.03	0.57*	0.41						
Na	-0.17	0.51	0.13	0.62*					
K	-0.23	0.53*	-0.06	0.36	0.77**				
Zn	-0.27	0.45	0.39	0.45	0.62*	0.65*			
Cu	-0.47	-0.09	0.61*	-0.10	0.01	0.13	-0.71**		
Fe	0.03	-0.08	0.17	0.06	-0.11	-0.37	0.02	-0.01	
Mn	-0.49	-0.26	0.30	-0.11	0.01	0.27	0.16	0.82**	0.07

* Correlation coefficients statistically significant at $p<0.05$

** Correlation coefficients statistically significant at $p<0.01$.

of all mineral elements, including heavy metals, in their meat, internal organs or in hair (FRENCH *et al.* 2017). Within the past several years there have been some considerable changes introduced with the object of limiting heavy metals emission. However, despite those restrictions and a constant improvement in the field of technology, their amount in the environment is still rising. They are being biomagnified in the alimentary chain and consumed by people along with products of plant and animal origin (DOBROWOLSKI 1995; KOŁACZ *et al.* 1996). Environmental pollution affects farm and wild animals equally (FALANDYSZ 1993; DROZD & KARPIŃSKI 1997). However, at the same time the latter are perfect bioindicators of the level of environmental contamination.

One of the elements under investigation found in the hair of red deer was lead, for which the highest concentration was observed in the samples from the Masovian Province, i.e. 10.16 mg/kg (Table 1), which may be a result, inter alia, of the high traffic density in this region, as research on the content of this element in the local air showed levels of 0.25 to 0.35 µg/m^3. The reason for this result may also be the stationary emissions from fossil-fuel power stations and technological sources (STATE OF THE ENVIRONMENT in the Masovian Province in 2015). A similar situation was observed in the Lesser Poland Province, where the mean concentration of lead in the hair of red deer was 9.46 mg/kg. The smallest amount of this element, i.e. 7.54 mg/kg, was observed in the samples from the Kuyavian-Pomeranian Province, which may be connected with the bigger forestation of this region and low concentration of lead in the air, reaching only 0.2 µg /m^3 (THE REPORT on the state of the environment Kuyavian-Pomeranian Province in the years 2013-2015).

The impact of location on the content of lead, inter alia, in the hair of horses was also demonstrated by KOŚLA *et al.* (1989), and in this case the concentration of this element was lower for the individuals from Mazowsze (Masovia) Region than for the ones from the southern part of Poland. The content of lead in the hair of other species was similar and dependent not only on their habitat, and in the case of farm animals on the feed and the time spent on the pasture (CIEŚLA & JANISZEWSKA 1999), but also on the animals' sex (BODAK *et al.* 1996; BUDZYŃSKI & TRUCHLIŃSKI 2004). Elevated levels of this element were found in the hair of horses from a stud in Michałowo. The content of lead in the hair of mares was as high as 0.94 mg/kg (BUDZYŃSKI & TRUCHLIŃSKI 2004). Similar research was conducted on stallions of Holsteiner, Polish Noble Half-bred and Ardennes breeds. No differences in the concentration of lead depending on the breed were noted, and its mean value was 0.136 mg/kg. It was, however, observed that during summer the level of this element rose, which is presumably associated with the animals staying longer in the paddock (CIEŚLA & JANISZEWSKA 1999). KUCHARCZAK *et al.* (2003) analyzed the hair of roe deer and wild boar from areas with different levels of environmental pollution: Wrocław and Legnica. The content of lead in the hair of roe deer from Wrocław and Legnica was 2.18 mg/kg and 1.92 mg/kg, respectively. As for wild boar, the content of lead in hair was 3.09 mg/kg for animals from the vicinity of Wrocław and 4.70 mg/kg for animals from the Legnica-Głogów Copper Region, and those values were half as high as in the research performed by the authors. The content of lead in the hair of both roe deer and wild boar was considerably higher than in other organs and tissues. The analyses of the content of lead in the hair of animals from those areas emphasized the "usability" of game animals in assessing the level of environmental pollution.

As our own research has revealed, the location of the animals' habitat in the context of the experimental material collected from the individuals has a profound effect on the concentration of the selected elements in their hair (Tables 1-3). This finds confirmation in the research by CYGAN-SZCZEGIELNIAK *et al.* (2014) concerning the concentrations of selected mineral elements in the hair of cattle from three provinces. Local environmental pollution had a great impact on the content of those elements in the samples. In our own research the content of cadmium in the hair of deer from Lesser Poland was higher by approximately 60% than in Masovian or Kuyavian-Pomeranian Provinces, and 33% higher than in the hair of cows under investigation by CYGAN-SZCZEGIELNIAK *et al.* (2014). The concentration of lead was in turn from 5 to 37 times higher in the hair of deer than in the hair of cows. The remaining trace elements showed similar concentrations for cows and deer, except for iron, the amount of which was 3-4 times higher in the hair of deer than was recorded in cows. It should be emphasized that the analysis of the presented results is difficult due to little information in the literature concerning the concentrations of those elements in the fur of wild animals. It is also complicated to make comparisons between farm and wild animals, as the latter are more susceptible to environmental influence. The research of FILISTOWICZ *et al.* (2011) conducted on the hair of farm and wild foxes confirms that the amount of the accumulated elements differs in both groups of animals. When compared to the hair of deer obtained in our own research, the hair of wild foxes from the area of Middle-West Poland showed, on average, a five-fold lower concentration of Pb, twice as low concentration of Cu, and a similar content of Zn. The comparison of the concentration of Pb in deer hair and other tissues and organs is also a noteworthy observation. In research by FALANDYSZ *et al.* (2005) the concentration of this element in the liver, kidneys and muscles was respectively 0.26, 0.36 and 0.21 mg/kg wet weight, and it was definitely lower than in hair in our own research. The content of cadmium in the hair of deer, regardless of the province of the animal's origin, was on average lower by a half than in the liver (0.23 mg/kg wet weight), and over a dozen times lower than in the kidneys (2.7 mg/kg wet weight) in the research by FALANDYSZ *et al.* (2005).

The mean content of cadmium in the hair of red deer was highest in the samples from the Lesser Poland Province, and it reached 0.18 mg/kg (Table 1). This may be related to industry, as within this province 24 factories of particular environmental nuisance are located, inter alia, Polish Steel Mills (THE REPORT on the state of the environment Lesser Poland Province in the years 2013-2015).

The level of cadmium in the hair of various animals reflects the individual's condition and the degree of environmental contamination. This is well illustrated by the research on the content of this element in the hair of horses (KRUPA *et al.* 2006). In the research on pure blood Arabian mares only, with a focus on genealogical lines, inter-individual variability was revealed. As the breeding and feeding conditions were unified, it was concluded that the differences stemmed from genetic factors. The mean level of cadmium in the hair of mares of the Shierife breed was 0.026 mg/kg, while for the Ibrahim breed this value was as little as 0.005 mg/kg (KRUPA *et al.* 2006). The research on the content of cadmium in the hair and organs of wild animals from the vicinity of Legnica and Wrocław shows that in roe deer the concentration reached the highest level in kidneys, ranging from 0.35 to 0.46 mg/kg. A similar situation was observed in wild boar, where in the kidneys of the animals this value oscillated between 1.33 and 2.22 mg/kg, and in hair from 0.07 to 0.12 mg/kg (KUCHARCZAK *et al.* 2003).

In the authors' own research the average content of calcium in the hair of red deer stayed in the range of 9.37 g/kg to 12.07 g/kg, depending on the province (Table 2). The results of four-times lower concentrations of calcium compared to our own research, i.e. 2.36-3.22 g/kg, were obtained by CYGAN-SZCZEGIELNIAK *et al.* (2012) in the hair of heifers, depending on the season and location. The concentration of this element in the tissues is primarily affected by its content in the feed. In the hair of goats fed with regular feed with no addition of minerals and herbs the average content of calcium was 1.42 g/kg, while the ones which obtained feed enriched with a mineral and vitamin mix showed the level of 1.57 g/kg of calcium content in hair (BIS-WENCEL 2003), which was a statistically important difference in comparison to the control group.

The mean content of magnesium in the hair of red deer differed only slightly between the provinces, and it ranged between 0.58 g/kg and 0.68 g/kg (Table 2). For comparison, the concentration of magnesium in the hair of heifers oscillated between 4.9 and 6.2 g/kg, depending on the season and region (CYGAN-SZCZEGIELNIAK *et al.* 2012), and in the hair of free ranging European bison this value was 6 times lower compared to our own results, reaching as little as 0.097 g/kg (KOŚLA *et al.* 2011). Other researchers also noted considerably lower results, in the case of bovine hair, i.e. from 0.015 g/kg to 0.063 g/kg (BUDZYŃSKA *et al.* 2006; GABRYSZUK *et al.* 2010; KRUPA & BUDZYŃSKA 2011).

The content of sodium in animal hair usually remains at the same level. This is well illustrated by the results of research on mares of pure Arabian blood with a focus on genealogical lines (KRUPA *et al.* 2006). In both cases of individuals descending from male or female lines the mean content of sodium in hair was 221.033 mg/kg. For a reference, in the hair of a gelding from a stud in Janowo Podlaskie the concentration of sodium was 896.1 mg/kg (BUDZYŃSKI & TRUCHLIŃSKI 2004). In the hair of red deer the content of sodium oscillated between 3 and 4 g/kg, which after calculating gave values several times higher than those presented above.

In our own research the mean content of essential trace elements in the hair of red deer showed statistically significant differences depending on the province (Table 3). It is worth emphasizing that the amount of the elements present in the animals' bodies is chiefly dependent on the soil-plant-animal alimentary chain (DOBROWOLSKI 1995). In the areas taken into account for this research the natural presence of zinc in amounts ranging from 23.3 to 32.4 mg/kg, as well as of copper (4.7-6.5 mg/kg) in soil was found (STATE OF THE ENVIRONMENT in the Masovian Province in 2015; THE REPORT on the state of the environment Lesser Poland Province in the years 2013-2015; THE REPORT on the state of the environment Kuyavian-Pomeranian Province in the years 2013-2015).

The content of Zn in the hair of red deer ranged between 114 and 158 mg/kg (Table 3). For comparison, in research of KUCHARCZAK *et al.* (2003) the hair of roe deer from two regions, Wrocław and Legnica-Głogów Copper Region, showed slightly lower levels of this element's concentration, i.e. 93.32 mg/kg and 98.17 mg/kg, respectively. Similar results were obtained from the analysis of the hair of wild boar from the same areas as the roe deer, and it was 44.57 mg/kg for animals from the vicinity of Wrocław, and 137.24 mg/kg for animals from the area near Grębocice. A lower concentration of zinc was observed in the animals' parenchymatous organs (kidneys – 28.46 mg/kg). However, in the wild boar from the Legnica-Głogów Copper Region, in all analyzed tissues, including hair, the concentration of this element was remarkably higher than in the animals from the area of Wrocław, and in hair it was even tripled, which can be calculated from the above numbers.

The average concentration of Cu in the hair of red deer was 18 mg/kg (Table 3). The presence of copper and its relation to feeding was demonstrated by the research by BIS-WENCEL (1999). Goats which were fed feed containing no copper displayed symptoms of its deficiency, and its concentration in hair was 5.48 mg/kg, which was twice as low as in the individuals whose feed contained mineral supplement, and three-fold lower than in the results of our own research (BIS-WENCEL 2003). For comparison, the content of copper in the hair of calves in their first six months of life is very varied, and it depends to a high degree on environmental conditions and the presence of mineral additions to the feed. The mean concentration of copper in the hair of calves was 8.36 mg/kg, and it showed dynamic changes depending on the time of collecting the sample (SABA *et al.* 1991; BOMBIK *et al.* 2003). A deficiency of copper was also observed in dairy cows from Central Pomerania (MONKIEWICZ *et al.* 1994). The mean concentration of this element in the animals' hair ranged from 5.5 to 6.84 mg/kg, and it was dependent on lactation, physiological condition, and the kind of obtained feed (ODÓJ *et al.* 2003). In a study on the effect of the environment on the content of copper in the hair of roe deer from two areas of Poland: Wrocław and the Legnica-Głogów Copper Region (KUCHARCZAK *et al.* 2003), a higher concentration of this element was noted in the vicinity of Grębocice, where the average value of this parameter was 5.8 mg/kg. In the area surrounding Wrocław the mean concentration of copper in the hair of roe deer was 4.13 mg/kg. A similar situation was observed in the case of wild boar. However, the difference between the content of copper in the hair of the animals from those two areas was 5-fold, as in the individuals from the Legnica-Głogów Copper Region it was 10.11 mg/kg, while in the vicinity of Wrocław it was as little as 1.98 mg/kg (KUCHARCZAK *et al.* 2003). In other game species, i.e. hare, the mean content of copper in hair was 0.13 mg/kg (DŁUGASZEK & MULARCZYK-OLIWA 2004).

In our own research the lowest concentration of iron in the hair of red deer was noted in the samples from the Kuyavian-Pomeranian Province (Table 3). This result may be associated with the soil in this area, as in this province it is slightly more fertile and less polluted than in the other two provinces taken into account in this study. Also, in the case of this element its supplementation in animals' feed has a great impact on its distribution in the organism. Support for this thesis may be provided by research on the hair of goats of the white refined breed, in which an increase in the concentration of iron was observed after 10 weeks of adding a mineral and herb mix to their feed, i.e. from 121.8 mg/kg to 136.9 mg/kg (BIS-WENCEL 2003). The presence of the mix in the feed also affected the calves of the black and white breed. In this research concentrations of iron in hair ranging from 100.0 to 127.5 mg/kg was observed. The lowest amount of this essential trace element was noted in the control group, fed with regular feed with no additions (BOMBIK *et al.* 2001). Among game animals, the content of iron in hair was determined, inter alia, in hare, and it was 12 mg/kg (DŁUGASZEK & MULARCZYK-OLIWA 2004).

In our own research the lowest concentration of manganese in hair, 13.87 mg/kg, was noted in red deer from the Masovian Province, and the highest, 19.27 mg/kg, in animals from the Kuyavian-Pomeranian Province (Table 3). For comparison, in the hair of calves the concentration of manganese ranged between 19.31 mg/kg and 26.08 mg/kg, and it was affected by the kind of mineral and herb addition to the feed, as well as by the month of life of the individual (BOMBIK *et al.* 2001).

Research on the content of particular elements in hair or fur provides information concerning the general level of environmental contamination, as well as animal or human health condition. It is not uncommon that concentrations of heavy metals in animal hair reach higher levels than in other tissues (KUCHARCZAK *et al.* 2003). The analysis of correlation showed positive and statistically important relations between a number of elements, among which the most significant occurred in the pairs Na-K and Cu-Mn (Tab. 4). The analysis of mineral content of the hair of heifers from Kietrz, Knyszyn and Osięciny (CYGAN-SZCZEGIELNIAK *et al.* 2012) also showed positive and statistically important correlations between those elements. In the presented paper only one negative and statistically significant relationship was observed, between Cu and Zn (Table 4). The research by KABATA-PENDIAS & PENDIAS (1999) proved a metabolically significant antagonism between Zn and Cu as well, where the elements compete with each other for their absorption from the alimentary tract. Similar interactions between the elements in the hair of dairy cows were observed by BUDZYŃSKA *et al.* (2006). The presence of Cu directly affects the level of Zn in the organism and its metabolism (BÀRÀNY *et al.* 2002; LOPEZ-ALONSO *et al.* 2005). There is an antagonism between the ions of Cu and Zn (BREMNER & BEATTIE 1995), and a synergism between the ions of Cu and Fe, which has a particularly beneficial effect on various enzymatic processes, especially during synthesis of hemoglobin.

An interesting study was also conducted by GIŻEJEWSKA *et al.* (2017) on the content of selected mineral elements and heavy metals in the antlers of deer from the area of North-Eastern Poland. In this paper the content of Cu in the antlers of deer was 6 times lower than in the fur in our study, while the content of Zn was similar to that recorded in the hair of deer from the Masovian Province and lower by 50% than in the hair of deer from the remaining provinces. A comparable content between the antlers and hair was also found in the case of iron, regardless of the location the samples were collected from. It is noteworthy that a tenfold higher concentration of cadmium and an approximately 21 times higher concentration of lead was found in the hair when compared to the antlers. In the case of iron, as revealed by SZKODA *et al.* (2004), its concentration in the liver of wild animals ranges on average from 3.64 to 170.3 mg/kg, and in kidneys from 51.81 to 200 mg/kg. When taking into account the results of our own research, the values obtained from hair were significantly higher for this element than in those organs and were similar to the ones reported by GIŻEJEWSKA *et al.* (2017) in antlers, i.e. 220 mg/kg.

In conclusion, the larger content of mineral substances and heavy metals in comparison to farm animals proves the usability of wild animals for the purposes of the assessment of environmental pollution. The measurement of the concentration of those elements in the hair of red deer (*Cervus elaphus* L.) may serve as a perfect alternative to other methods for the evaluation of the level of environmental contamination.

Author Contributions

Research concept and design: D.C.-S., B.J.; Collection and/or assembly of data: D.C.-S., K.S., B.J.; Data analysis and interpretation: D.C.-S., M.S.; Writing the article: D.C.-S.; Critical revision of the article: D.C.-S., M.S., K.S., A.R.; Final approval of article: D.C.-S., M.S., A.R.

Conflict of Interest

The authors declare no conflict of interest.

References

BÀRÀNY E., BERGDAHL I.A., BRATTEBY L.E., LUNDH T., SAMUELSON G., SCHUTZ A., SKERFVING S., OSKARSSON A. 2002. Relationships between trace element concentrations in human blood and serum. Toxicol. Lett. **134**: 177-184.

BIS-WENCEL H. 1999. Indices of mineral metabolism in goats from South-Eastern Poland. Ann. UMCS sect. EE **17**: 353-360.

BIS-WENCEL H. 2003. Effect of a herbal-mineral mixture on indicators of goat mineral metabolism. Ann. UMSC sect. EE **21**: 345-353.

BODAK E., KOŁACZ R., DOBRZAŃSKI Z. 1996. Heavy metals – the conditions of exposure and defense mechanisms in animals. Medicine Vet. **52**: 619-625.

BOMBIK T., BOMBIK E., SABA L. 2001. Wpływ mieszanek mineralno-ziołowych na poziom wybranych mikroelementów w sierści cieląt (The effect of mineral-herb mixtures on the level of selected microelements in the hair of calves). Folia. Univ. Agric. Stetin., Zoot. **42**: 17-22. (In Polish with English summary).

BOMBIK E., BOMBIK A., SABA L. 2003. Wpływ mieszanek mineralno-ziołowych na poziom miedzi, kobaltu i molibdenu w sierści cieląt (The effect of mineral-herb mixtures on the level of copper, cobalt and molybdenum in the hair of calves). Zesz. Nauk. Prz. Hod. **67**: 213-220. (In Polish with English summary).

BREMNER I., BEATTIE J.H. 1995. Copper and zinc metabolism in health and disease: speciation and interactions. Proc. Nutr. Soc. **54**: 489-499.

BUDZYŃSKA M., KRUPA W., SOŁTYS L., SAPUŁA M., KAMIENIAK J., BUDZYŃSKI M. 2006. Poziom biopierwiastków w sierści krów mlecznych (Level of bio-elements in dairy cows' hair). Ann. UMCS sect. EE **24**: 327-333. (In Polish with English summary).

BUDZYŃSKI M., TRUCHLIŃSKI J. 2004. Ocena składu biopierwiastków zawartych w organizmie koni na podstawie analizy ich zawartości w sierści (The assessment of content of dietary minerals in horses, based on the analysis of their levels in the animals' hair). Ann. UMCS sect. EE **22**: 253-261. (In Polish with English summary).

CHATT A., KATZ S. 1989. Hair analysis: application in the biomedical and environmental science. New York: VHC Publishers Inc. 1-16.

CHO S.Y. 1997. Trace element exposure in man by instrumental neutron activation analysis of hair. J. Radioanal. Nucl. Ch. **217**: 107-109.

CIEŚLA A., JANISZEWSKA J. 1999. Concentration of cadmium and lead in the hair of half-breed horses depending on the breeding center and season. Folia. Univ. Agric. Stein., Zootech. **37**: 23-30.

CYGAN-SZCZEGIELNIAK D., STANEK M., GIERNATOWSKA E., JANICKI B., GEHRKE M. 2012. Zawartość wybranych składników mineralnych w sierści jałówek w zależności od regionu hodowli i pory roku (Content of selected mineral elements in heifer hair depending on the region and season). Medicine Vet. **68**: 293-298. (In Polish with English summary).

CYGAN-SZCZEGIELNIAK D., STANEK M., GIERNATOWSKA E., JANICKI B. 2014. Impact of breeding region and season on the content of some trace elements and heavy metals in the hair of cows. Folia Biol. (Kraków) **62**: 163-169. DOI: https://doi.org/10.3409/fb62_3.163

DECREE OF THE MINISTRY OF ENVIRONMENT from 16 March 2005 concerning the allowed periods of game hunting. Journal of Laws 2005 no 48 item 459. http://prawo.sejm.gov.pl/isap.nsf/DocDetails.xsp?id=WDU20050480459

DŁUGASZEK M., MULARCZYK-OLIWA M. 2004. The analysis of content of dietary minerals and toxic metals in the hair of small game animals and game ruminants. J. Elementol. **9**: 263-268.

DOBROWOLSKI J. 1995. Circulation of elements in the environment. Materials of the 1st International Conference in Warsaw, pp 14.

DROZD L., KARPIŃSKI M. 1997. Zawartość metali ciężkich w tkankach jeleni i danieli pochodzących z chowu fermowego (The content of heavy metals in the tissues of red deer and fallow deer bred on farms). Ann. UMCS sect. EE **15**: 309-314. (In Polish with English summary).

DUNNETT M., LEES P. 2003. Trace element, toxin and drug elimination in hair with particular reference to the horse. Res. Vet. Sci., **75**: 89-101.

FALANDYSZ J. 1993. Metale w tkance mięśniowej dziczyzny na terenie północnej części kraju (Metals in the muscle tissue of game animals in the northern parts of Poland). Bromatol. Chemia Toksykol. **26**: 205-206. (In Polish).

FALANDYSZ J., SZYMCZYK-KOBRZYŃSKA K., BRZOSTOWSKI A., ZALEWSKI K., ZASADOWSKI A. 2005. Concentrations of heavy metals in the tissues of red deer (*Cervus elaphus* L.) from the region of Warmia and Mazury, Poland. Food Addit. Contam. **22**: 141-149.

FILISTOWICZ A., DOBRZAŃSKI Z., PRZYSIECKI P., NOWICKI S., FILISTOWICZ A. 2011. Concentration of heavy metals in hair and skin of silver and red foxes (*Vulpes vulpes*). Environ. Monit. Assess. **182**: 477-484. DOI: https://doi.org/10.1007/s10661-011-1891-3

FRENCH A.S., SHAW D., GIBB S.W., TAGGART M.A. 2017. Geochemical landscapes as drivers of trace and toxic element profiles in wild red deer (*Cervus elaphus*). Sci. Total Environ. **601-602**: 1606-1618. DOI: https://doi.org/10.1016/j.scitotenv

GABRYSZUK M., SŁONIEWSKI K., METERA E., SAKOWSKI T. 2010. Content of mineral elements in milk and hair of cows from organic farms. J. Elementol. **15**: 259-267. DOI: https://doi.org/10.5601/jelem.2010.15.2.259-267

GIŻEJEWSKA A., SZKODA J., NAWROCKA A., ŻMUDZKI J., GIŻEJEWSKI Z. 2017. Can red deer antlers be used as an indicator of environmental and edible tissues' trace element contamination? Environ. Sci. Pollut. Res. **24**: 11630-11638. DOI: https://doi.org/10.1007/s11356-017-8798-7

GRATACÓS-CUBARSÍ M., CASTELLARI M., VALERO A., GARCÍA-REGUEIRO J.A. 2006. Hair analysis for veterinary drug monitoring in livestock production. J. Chromatogr. B. **834**: 14-25. DOI: https://doi.org/10.1016/j.jchromb.2006.03.007

JAUHARAH M.D.K., HUSIN W., HOSSAIN I., NOORDDIN I. 2013. Screening heavy metals levels in hair of sanitation workers by X-ray fluorescence analysis. J. Environ. Radioact. **115**: 1-5. DOI: https://doi.org/10.1016/j.jenvrad.2012.05.013

KABATA-PENDIAS A., PENDIAS H. 1999. Biogeochemia pierwiastków śladowych (Biogeochemistry of trace elements). Scientific Publishing PWN, Warszawa. Edition 2. 400pp. (In Polish).

KOŁACZ R., DOBRZAŃSKI Z., BODAK E. 1996. The bioaccumulation *Cd, Pb and Hg* in the tissues of *animals.* Medicine Vet. **52**: 686-691.

KOŚLA T., ANKE M., GRÜN M. 1989. The lead status of horses from central Europe depending on breed, sex, age and living area. Arch. Tierernahr. **39**: 667-674. DOI: https://doi.org/10.1080/17450398909428336

KOŚLA T., SKIBNIEWSKA E.M., SKIBNIEWSKI M. 2011. The state of bioelements in the hair of free-ranging European bisons from Białowieża Primeval Forest. Pol. J. Vet. Sci. **14**: 81-86. DOI: https://doi.org/10.2478/v10181-011-0012-0

KRUPA W., BUDZYŃSKA M. 2011. Zawartość wybranych biopierwiastków w sierści krów mlecznych w zależności od niektórych parametrów produkcji mlecznej (Influence of certain parameters of milk production on the content of selected bio-elements in cows' hair). Medicine Vet. **67**: 332-334. (In Polish with English summary).

KRUPA W., SOŁTYS L., BUDZYŃSKA M., SAPAŁA M., KAMIENIAK J., BUDZYŃSKI M. 2006. Ocena skladu mineralnego siersci klaczy czystej krwi arabskiej z uwzglednieniem linii genealogicznych (Assessment of mineral content in Purebred Arabian mares' hair considering genealogical lines). Ann. UMCS sect. EE **24**: 209-216. (In Polish with English summary).

KUCHARCZAK E., JOPEK Z., MORYL A. 2003. Wpływ środowiska na zawartość wybranych metali (Pb, Cd, Zn,Cu) w tkankach saren i dzików. (The impact of the environment on the content of selected metals (Pb, Cd, Zn, Cu) in the tissues of roe deer and wild boar). Acta Sci. Pol. Med. Vet. **2**: 37-47. (In Polish with English summary).

LOPEZ-ALONSO M., PRIETO F., MIRANDA M., CASTILLO C., HERNÁNDEZ J.R., BENEDITO J.L. 2005. Intracellular distribution of copper and zinc in the liver of copper-exposed cattle from northwest Spain. Vet. J. **170**: 332-338. DOI: https://doi.org/10.1016/j.tvjl.2004.07.007

MICHALAK I., MIKULEWICZ M., CHOJNACKA K., WOŁOWIEC P., SAEID A., GÓRECKI H. 2012. Exposure to nickel by hair mineral analysis. Environ. Toxicol. Pharmacol. **34**: 727-734. DOI: https://doi.org/ 10.1016/j.etap.2012.09.015

MONKIEWICZ J., GERINGER H., NICPOŃ J. 1994. The impact of specific environmental threats on cows bred in the Copper Region of Poland. Medicine Vet. **50**: 162-166.

ODÓJ J., WNUK W., SABA L., BIS-WENCEL H., NOWAKOWICZ-DĘBEK B. 2003. The content of selected essential trace elements in the milk and hair of cows from Middle Pomerania. Ann. UMCS **21**: 339- 344.

PATKOWSKA-SOKOŁA B., DOBRZAŃSKI Z., OSMAN K., BODKOWSKI R., ZYGADLIK K. 2009. The content the chosen chemical elements in wool of sheep of different origins and breeds. Arch. Tierz. **52**: 410-418. DOI: https://doi.org/10.5194/aab-52-410-2009

POLISH STANDARD I.S. EN 13805: 2014. Foodstuffs. Determination of trace elements. Pressure digestion. https://infostore.saiglobal.com/preview/is/en/2014/i.s.en138052014.pdf?sku=1769739.

POLISH STANDARD PN-EN 14084: 2004. Foodstuffs. Determination of trace elements. Determination of lead, cadmium, zinc, copper and iron by atomic absorption spectrometry (AAS) after microwave mineralization. http://sklep.pkn.pl/pn-en-14084-2003e.html.

SABA L., BIAŁKOWSKI Z., WÓJCIK S. 1991. Badania nad zaopatrzeniem mineralnym krów w wybranych obiektach regionu południowo-wschodniej Polski (Studies on the mineral supply of cows in selected south-eastern region of Poland). Rocz. Nauk Rol. B **106**: 25-37. (In Polish).

STATE OF THE ENVIRONMENT in the Masovian Province in 2015. Stan środowiska w województwie mazowieckim w 2015 roku. Voivodeship Inspectorate for Environmental Protection in Warsaw, Warsaw 2016, pp. 1-135. https://www.wios.warszawa.pl/pl/publikacje-wios/publikacje/1266,Stan-srodowiska-w-wojewodztwie-mazowieckim-w-2015-roku.html. (In Polish).

SZKODA J., ŻMUDZKI J. 2001. Pierwiastki toksyczne w tkankach zwierząt łownych (Toxic elements in tissues of game animals). Medicine Vet. **57**: 883-886. (In Polish with English summary).

SZKODA J., ŻMUDZKI J., GRZEBALSKA A. 2004. Żelazo w tkankach zwierząt i mleku (Iron in animal tissues and milk). Rocz. Panstw. Zakl. Hig. Suppl. **55**: 61-6. (In Polish with English summary).

THE REPORT on the state of the environment Kuyavian-Pomeranian Province in the years 2013-2015. Raport o stanie środowiska województwa kujawsko-pomorskiego w latach 2013-2015. Voivodeship Inspectorate for Environmental Protection in Bydgoszcz, Library of Environmental Monitoring, Bydgoszcz 2016, pp. 1-89. http://www.wios.bydgoszcz.pl/publikacje/raporty. (In Polish).

THE REPORT on the state of the environment Lesser Poland Province in the years 2013-2015. Raport o stanie środowiska w województwie małopolskim w latach 2013-2015. Voivodeship Inspectorate for Environmental Protection in Cracow, Cracow 2016, pp. 1-120. http://www.krakow.pios.gov.pl/Press/publikacje/raporty/raport15/index.htm. (In Polish).

TYMCZYNA L., SABA L., KAMIENIECKI K., BIS-WENCEL H., WNUK W. 2000. Występowanie i rozpoznanie niedoborów i dysproporcji w gospodarce mineralnej u bydła mlecznego rejonu Pomorza Środkowego (Occurrence and recognition of deficiency and disproportions in the mineral management for milk cattle from the area of central Pomerania). Ann UMCS sect. EE **18**: 215-221. (In Polish with English summary).

WONGSASULUK P., CHOTPANTARATB S., SIRIWONGE W., ROBSONE M. 2018. Using hair and fingernails in binary logistic regression for bio-monitoring of heavy metals/metalloid in groundwater in intensively agricultural areas. Thailand. Environ. Res. **162**: 106-118. DOI: https://doi.org/10.1016/j.envres.2017.11.024