ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., Supplement 15, August 2020: 229-234

Biodiversity, Ecology and Conservation
Research Article

Proceedings of the Third International Conference on Zoology, Zoonoses and Epidemiology, Hissar, Bulgaria, October 21-23, 2019

http://www.acta-zoologica-bulgarica.eu/Suppl_15_41

Red deer *Cervus elaphus* L., 1758 (Mammalia: Cervidae) as a Biomonitor for Contemporary Heavy Metal Pollution of the Environment in Forest Mountain Regions in Bulgaria

Georgi G. Markov[1,], Atidzhe A. Ahmed[1] & Chavdar D. Zhelev[2]*

[1] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria; E-mail: georgimar@gmail.com; atidje_dj@abv.bg
[2] Southwestern State Enterprise, 18 Zora Street, Blagoevgrad, Bulgaria; email: chachko17@abv.bg

Abstract: The aim of the study was to check the current levels of toxic (Pb and Cd) and essential with a concentration-dependent toxic effect (Cu and Zn) heavy metals in the liver of the European red deer *Cervus elaphus* inhabiting typical forest mountain regions in Bulgaria, Rhodope Mountains and in Central Stara Planina Mountains. For determination of analytic concentrations of the studied heavy metals, an inductively coupled plasma atomic emission spectrometry (ICP-AES) was used. The average values of metal concentration (mg/kg dry weight) in the liver of the red deer from Rhodope Mountains were as follow: Cu – 176.7, Zn – 599.3, Pb – 2.2 and Cd – 0.9; in Central Stara Planina Mountain, they were: Cu – 115.6; Zn – 224.7; Pb – 5.0 and Cd – 0.9. The obtained results can be used to create a baseline for estimation of the current heavy metal accumulation in red deer in the feeding area of the big game animals in the forest mountain regions of Bulgaria. They also offer an opportunity that red deer be used as a biomonitor of future potential anthropogenic negative impact on the environment in forest regions of the country under the conditions of modern anthropogenic activities therein.

Key words: Rhodope Mountains, Central Stara Planina Mountain, environmental pollution

Introduction

The red deer *Cervus elaphus* L., 1758 is widespread in the Holarctic and Nearctic. Based on coat colour and morphological characters of skulls and antlers, 22 subspecies have ben recognised within this species (Ellerman & Morrison-Scott 1966, Grubb 2005). In present days, red deer is one of the biggest free-ranging mammals of Europe. Its distribution range comprises France, the Netherlands, Belgium, Luxembourg, Denmark, Switzerland, Germany, Austria, Czech Republic, Italy, Balkan states, Poland and the western part of the former Soviet Union (Hartl et al. 2003). The environment on the territory of Bulgaria, both in historical times (Spassov & Iliev 1994) and in nowadays (Markov 1959, Dragoev 1978, Botev 1985) offers suitable conditions for the red deer populations. Currently, its distribution in Bulgaria is concentrated mainly in biotopes in the Central and Eastern Stara Planina, Central Sredna Gora, Rila-Rhodopes and Strandzha-Sakar mountain areas, Kraishte-Osogovo and Ludogorie-Dobrudzha regions (Stenin 2008). Therein, it inhabits not very dense deciduous and mixed forests in flat, semi-mountainous and mountainous areas but often its habitats are also in semi-open plain areas. Although in the recent past red deer were rare in parts of Bulgaria and close to extinction, due to

[*]Corresponding author: georgimar@gmail.com

the reintroduction and conservation efforts such as re-acclimatization and breeding in farms (Dragoev 1978), its number has reached 28,370 individuals in 1992; in 2012, there were almost 21,000 individuals (AEFA 2012). Nowadays, according to the state statistics for 2018, the total number of red deer is 29,331 individuals in Bulgaria (Dzhuparov 2018). In Bulgaria (Stenin 2008, Obretenov 2010), as in other European countries (Apollonio et al. 2010), the red deer is one of the key species of terrestrial ecosystems and the main hunting species of great importance in the forest mountain areas. Red deer has browsers feeding on grasses, sedges and forbs in summer. In the winter, they eat woody growth, so they have an important ecosystem role in shaping the plant communities in which they live through their browsing. Young individuals may also serve as an important source of prey for large predators.

The increased environmental pollution by heavy metals leads to their accumulation in the wildlife (Goyer 1986). The real risk presented by heavy metals to wildlife and humans is mostly demonstrated as a chronic or sub-lethal effect, e.g. nephrotoxicity, carcinogenicity, teratogenicity, endocrine and reproductive toxicities (Rani et al. 2014). The main threats to wildlife health from heavy metals are associated with exposure to non-essential elements such as lead (Pb) and cadmium (Cd); however, the anthropogenic pollution of ecosystems with essential heavy metals zinc (Zn) and copper (Cu) is also problematic at some sites. The latter two metals can be toxic to wildlife if their concentrations are high (Environmental Fact Sheet 2005).

Determinations of heavy metals in metabolically-active organs of selected wild mammalians (including hunted animals) serving as biomonitors can be used for the indirect assessment of conditions in terrestrial ecosystems. As accumulative biomonitors of environment pollution, they possess a number of merits such as great availability, low costs, retrospection, no servicing and consideration of synergistic/antagonistic effects, area-related results, biological relevance and comparability to man (Witting 1993). Wild animals, especially game species, with regular farming activities such as red deer are suitable as bioindicators (Froslie et al. 2001) due to their large geographical distribution, residential habits, relatively long lifespan and relatively easy sample collection. For sustainable use of the potential of red deer as a natural resource and for balanced development of terrestrial ecosystems, it is crucially necessary to assess the degree of load of their livers with toxic elements (Tataruch & Kierdorf 2003).

The aim of the present study was to check the load of red deer liver with heavy metals including not essential elements such as Pb and Cd as well as with essential metals with a concentration-dependent toxic effect such as Zn and Cu. The established in the current survey reference values of their residuals in red deer from forest mountain areas would give an opportunity to use this species as a bioindicator of future potential anthropogenic negative influence on the environment in forest mountain areas in the country in conditions of modern industrial activities.

Materials and Methods

Study area

The red deer included in this study were legally hunted during the hunting season of 2017/2018 from different areas in forest mountain regions in the Rhodope Mountains and in Central Stara Plan-

Fig. 1 Map of the study area. 1 – Central Stara Planina Mountain; 2 – Rhodope Mountains.

ina Mountains (Fig. 1). Both regions were characterised by relatively non-intense industrial development and poorly developed road network. The study area in the Rhodope Mountains represents a forest mountain region characterised by trophic areas of the big game, in which woody vegetation is varying and are located at altitudes up to about 1200 m. The natural prerequisites for the studied territory to be characterised by the presence in it of a number of hunting economic species of mammals such as red deer, mouflon, roe deer and wild boar are the diversity of terrain, wood, shrub and grass vegetation and the abundance of spring and river water. The habitats of the studied red deer from Central Stara Planina Mountain have a typical mountainous relief dominated by deciduous forests with a basic wood species – European beech (*Fagus sylvatica*). In them, besides the red deer, suitable conditions for development also find the roe deer, wild boar and predators.

Sampling methods

To avoid possible influence of age and sex of the studied individuals on the quantitative accumulation of the tested metals in their liver, only middle-aged (age between 5 and 6 years) male animals were investigated. The sex of the tested individuals was determined by an external examination; the age of each specimen was determined by assessing the state of the tooth system (Habermehl 1975). According to their initial veterinary examination, all investigated red deer were in normal physiological condition.

Liver samples from 10 red deer from Central Stara Planina Mountain and from 11 individuals from Rhodope Mountains were taken in the field. None of the samples were contaminated with bullets and pellets. The collecting of the biological material from red deer was carried out according to the methodological recommendations for the use of mammals as zoomonitor for the purposes of environmental monitoring of the environment, noted in the National Program for Biomonitoring of Bulgaria (1999). The collected samples were cooled in a portable cooler, transported to the laboratory and stored at −20°C freezer until analysis.

The preliminary preparation of samples for chemical analysis included the following steps: (a) drying to air dry weight and grinding to a homogenised mass, (b) dissolving the sample with a mixture of HNO_3 and $HClO_4$ for 24 hours at room temperature, (c) evaporating it to a wet residue on a sand bath and (d) quantitatively transferring it to a test tube and bringing it to a standard final volume with 1N HNO_3. The content of Pb, Cd, Zn and Cu in the analysed samples was determined using an inductively coupled plasma atomic emission spectrometry (ICP-AES) in a Perkin Elmer Optima 7000 DV instrument. The residual amounts of the studied elements were established using an atomic-absorption analysis. Concentrations of elements in the liver tissues were expressed as mg/kg of dry weight.

Statistical methods

Before analyses, the established concentrations of the studied heavy metals in the liver of the red deer inhabited both regions were tested for normality using Kolmogorov-Smirnov D-statistics and for homogeneity of variances using Levene's test. Concentrations of residuals of the heavy metals in the liver inhabiting Central Stara Planina Mountain were not normally distributed.

Hereof, the reference interval of the residues of all investigated heavy metals in animal's liver from both investigated habitats was calculated using a non-parametrical percentile method. All concentrations were described by their basic descriptive statistics (Mean, Median and Standard deviation). They were calculated by the algorithm of statistical analysis "Nonparametric Methods". The range of each variable was determined by the values of the 97.5th and 2.50th percentiles – width of the range about the median that includes 95% of the cases.

To check the significance of differences of the observed concentrations of the studied heavy metals in the liver's samples from the individuals from both habitats, a nonparametric test (Man-Whitney test) for independent samples was applied. Differences were considered as significant at the level of $p<0.05$.

To reveals the specificity of the heavy metals' residuals obtained in the liver of the red deer inhabiting the mountain landscapes in Bulgaria, the index Overall Metal Concentration according to Usero et al. (1996):

$$MPI = (Cf_1 x Cf_2 \ldots . Cf_n)^{1/n}$$

where Cf_n is the concentration of metal expressed in the sample was calculated. It was considered in a comparative aspect with those established for the same elements in the liver of the roe deer (Markov & Ahmed 2019a, 2019b) inhabiting the same mountain massifs in the same time and whose analytical concentration has been established by the same methodical approach. Statistical analysis of the data was performed using Statistica, V. 10 (StatSoft Inc. 2011).

Table 1. Values of basic statistical characteristics of the concentrations [mg/kg dry weight] of Zn, Cu, Pb and Cd in liver and metal pollution index (MPI) of adult male red deer inhabiting mountain landscapes in Bulgaria.

Metal	Basic statistical characteristics [mg/kg dry weight]			Limits of 95% range of the median [mg/kg dry weigh]		MPI
	Mean	Median	SD	From	To	
Rhodope Mountains						21.38
Cu	176.7	168.9	88.4	68.8	296.1	
Zn	599.3	652.2	306.4	104.3	987.5	
Pb	2.2	1.7	1.5	0.5	4.4	
Cd	0.9	0.5	0.9	0.2	3.2	
Central Stara Planina Mountain						18.48
Cu	115.6	116.7	59.8	20.8	196.9	
Zn	224.7	191.7	93.4	123.4	382.7	
Pb	5.0	4.7	1.2	3.2	6.8	
Cd	0.9	0.9	0.3	0.5	1.3	

Results

For the descriptive statistics for Pb, Cd, Zn and Cu concentration and metal pollution index (MPI) in the liver of the investigated red deer from the two mountain regions in Bulgaria, see Table 1. The results of the Man-Whitney test showed that the differences of the medians of the observed heavy metals' concentrations in red deer's liver from both target organs were of different statistical significance: for Cu, $p = 0.212$; for Zn, $p = 0.044$; for Pb, $p = 0.045$; for Cd, $p = 0.302$. As could be seen, established concentrations of zinc and lead were specific to each one in both regions, while the median value of the concentrations of copper and cadmium do not differ at p-value, which was treated as a „borderline acceptable" error level.

Discussion

The concentrations of the tested metals in the liver, actual for each mountain region, characterised by the width of the range about the median that includes 95% of the cases demonstrate the limits of their variability. Typical for the established intervals is that they have wide limits in both regions. The formal reason for the broad concentration limits is that in the liver of some individuals, the studied metals are of high value. This effect is probably related to the heterogeneity of the load on the separate components of the mountains environment because both of natural geochemical characteristics and human activities which greatly impair the natural environment in many parts of country. In the Rhodope Mountains, in the areas of mining much of the land is degraded. Flotation activity and lead-zinc extraction conduce to local contamination of the soil and water by heavy metals in non-ferrous metal production areas (Kopralev et al. 2002).

It was also found that red deer's liver form both investigated mountain regions were characterised by a different Metal Pollution Index. The mean MPI value of the red deer's liver from the Rhodope Mountains is 1.16 times higher than that from the Central Stara Planina Mountains. The overview of the MPI value of the red deer's liver with those of the roe deer inhabiting the same mountain massifs in the same time (MPI in the Rhodope Mountains is 12.96 and in the Central Stara Planina Mountains is 10.16) shows that their values in the red deer are considerably higher than those in the roe deer (Markov & Ahmed 2019a, 2019b).

The transfer of trace metals to terrestrial mammals depends on a number of factors the most important of which are: the species, its age and specialised of food availability (Hunter et al. 1987). The higher concentration of metals found in red deer liver than in roe deer liver may result from differences in their feeding preferences and metabolism (Prins & Geelen 1971), digestion rate and feeding frequency (Holand 1994) and anatomy of the digestive tract (Kottferová & Koréneková 1998). It should be noted that the comparative analysis of the heavy metal residues found in this study with results found in other studies across the country and Europe may have differences due to the fact that they did not take into account age and sex of the animals in the data processing, as well as in the calculation of the data related to the wet weight.

The presence of lead and cadmium, elements with proven highly toxic effect on living organisms in considerable concentrations, together with the noteworthy presence of elements with dependent toxic effects elements (copper and zinc) in sizeable residues in the liver of some red deer, requires concentrations of these elements in wild animals to be constantly monitored. This suggests that regular assessment and forecasting of accumulation of heavy metals in free-living game animals in ecosystems is necessary. Our study also reinforces the need to account carefully for specific environmental related variation in ecotoxicological research on wild-living animals in regions of the country characterised by specific ecological conditions as well as the potential for deterioration of the quality of the environment in the mountainous habitats, because of anthropogenic activity. Summing up it can be stated that: (1) the results create a baseline for the estimation of current heavy metal accumulation in red deer in the investigated regions and (2) they offer an opportunity this species to be used as a bioindicator of future potential anthropogenic negative impact on the environment in mountain forest regions in Bulgaria.

Acknowledgments: This work was supported by project № 195/10.10.2016 "Population studies, migration and status of the red deer (*Cervus elaphus*) in Bulgaria" of the Southwestern State Enterprise, Blagoevgrad and the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. We thank the staff of the State Hunting Enterprise for their cooperation and contribution.

References

AEFA 2012. Game numbers in Bulgaria. Archive of the Executive Forest Agency of Ministry of Agriculture and Foods of R. Bulgaria, Sofia. (In Bulgarian)

Apollonio M., Andersen R. & Putman R. 2010. European ungulates and their management in the 21st century. Cambridge, New York: Cambridge University Press. 618 p.

Botev N. 1985. Hunting economy. Sofia: Zemizdat. 270 p. (In Bulgarian)

Dragoev P. 1972. Enrichment of hunting fauna in Bulgaria. Sofia: Zemizdat. 102 p. (In Bulgarian)

Dzhuparov M. 2018. Overview of Game Stocks in Bulgaria. Forest 6-7: 18-20. (In Bulgarian)

Ellerman J.R. & Morrison-Scott T.C.S. 1966. Checklist of Palearctic and Indian Mammals 1758 to 1946. London: Trustees of the British Museum of Natural History. 810 p.

Environmental Fact Sheet 2005. Copper: Health Information Summary: Chronic (long-term) copper exposure can damage the liver and kidneys. New Hampshire Department of Environmental Services, ARD-EHP-9 2005. http://des.nh.gov/organization/commissioner/pip/factsheets/ard/documents/ardehp9 pdf

Froslie A., Sivertsen T. & Lochmiller R. 2001. Perissodactyla and Artiodactyla. In: Shore R. & Rattner F. B. A. (Eds.): Ecotoxicology of Wild Mammals. Chichester, England: John Wiley & Sons Ltd., pp. 497–550.

Goyer R.A. 1986. Toxic effects of metals. In: Klaassen C. D., Amdur M. & Doull J. (Eds.): Casarett and Doull's toxicology. The basic science of poisons. New York: Macmillan Publishing Company, pp. 582–635.

Grubb P. 2005. Order Artiodactyla. In: Wilson D.E. & Reeder D.M. (Eds.): Mammal species of the world. A taxonomic and geographic reference. Baltimore: The John Hopkins University Press, pp. 637–722.

Habermehl K.H. 1975. Die Altersbestimmung bei Haus und Labortieren. Berlin and Hamburg: Paul Parey. 216 p.

Hartl G.B., Zachos F. & Nadlinger K. 2003. Genetic diversity in European red deer (*Cervus elaphus* L.): anthropogenic influences on natural populations. Comptes Rendus Biologies 326 (1): 37–42.

Holand Ø. 1994. Seasonal dynamics of digestion in relation to diet quality and intake in European roe deer (*Capreolus capreolus*). Oecologia 98 (3–4): 274–279.

Hunter B.A., Johnson M.S. & Thompson D.J. 1987. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. III. Small Mammals. Journal of Applied Ecology 24(2): 601–614.

Kopralev I., Jordanova M. & Mladenov H. 2002. Geography in Bulgaria. Physical geography. Socio-economic geography. Sofia: Publishing House Forkom. 760 p. (In Bulgarian)

Kottferová J. & Koréneková B. 1998. Distribution of Cd and Pb in the tissues and organs of free-living animals in the territory of Slovakia. Bulletin of Environmental Contamination and Toxicology 60 (1):171–176.

Markov G. 1959. Mammals in Bulgaria. Sofia: Science and Art Publisher. 155 p. (In Bulgarian)

Markov G. & Ahmed A. 2019a. European roe deer (*Capreolus capreolus*) as a biomonitor for contemporary heavy metal pollution of the environment in forest mountain regions in Rhodope Mountains, Bulgaria. Ecologia Balkanica 10(2): 223–227.

Markov G. & Ahmed A. 2019b. Heavy metal residues in internal organs of roe deer (*Capreolus capreolus*) as a bioindicator of forest environmental contamination in West Stara Planina (West Bulgaria). Annual of Sofia University "St. Kliment Ohridski" Faculty of Biology Book 4 (Scientific Sessions of the Faculty of Biology 2019) 104: 308–314.

National Biomonitoring Program of Bulgaria. 1999. Peev D. & Gerasimov S. (Eds.): Sofia, Gaya-Libris. 237 p.

Obretenov A. 2010. Game breeding. Sofia: Nova Zvezda Publisher. 536 p. (In Bulgarian)

Prins R. A. & Geelen M.J.H. 1971. Rumen characteristics of red deer, fallow deer, and roe deer. Journal of Wildlife Management 35(4): 673–680.

Rani A., Kumar A., Lal A. & Pant M. 2014. Cellular mechanisms of cadmium-induced toxicity: A review. International Journal of Environmental Health Research 24(4): 378–399.

Spassov N. & Iliev N. 1994. Animal remains from the submerged Late Eneolithic – Early Bronze Age settlement near Sozopol (The South Black Sea Coast of Bulgaria). In: Lazarov M. & Angelova Ch. (Eds.): La Thrace et les sociétés maritimes anciennes, 18-24 September, 1994. Sozopol. Tracia Pontica. Proceedings of the International SymposiumVI., Series 1. Sozopol, 287–314.

STATISTICA (Data analysis software system), Vers. 10. Computer software. www.statsoft.com.

Stenin G. 2008. Hunting economy in Bulgaria. Sofia: Education and science Publisher. 296 p. (In Bulgarian).

Tataruch F. & Kierdorf H. 2003. Mammals as biomonitors. In: Markert B. A., Breure A. M. & Zechmeister H. G. (Eds.): Trace metals and other contaminants in the environment. Bioindicators & Biomonitors: Principles, Concepts and Applications, vol. 6. Amsterdam: Elsevier, pp. 737–772.

Usero J., González-Regalado E. & Gracia I. 1996. Trace metals in the bivalve mollusc *Chamelea gallina* from the Atlantic coast of southern Spain. Marine Pollution Bulletin 32 (3): 305–310.

Witting R. 1993. General aspects of biomonitoring heavy metals by plants. In: Markert B. (Ed.): Plants as biomonitors. Indicators for heavy metals in the terrestrial environment. VCH, Weinheim, pp. 3–27.