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HEAVY METAL RESIDUES IN INTERNAL ORGANS OF ROE DEER (*Capreolus capreolus*) AS BIOINDICATORS OF FOREST ENVIRONMENTAL CONTAMINATION IN WESTERN STARA PLANINA

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Abstract: The aim of the study was to check the current loading by heavy metals (Pb, Cd, Cu and Zn) of European roe deer (*Capreolus capreolus*), inhabiting typical forest mountain region in Western Bulgaria. The roe deer liver and kidneys were used as target organs for determination of analytic concentrations of them by an inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Perkin Elmer Optima 7000 DV. The concentrations of the tested metals, specific for each organ, showed a range of variability of median values. In the liver, the variability range of element concentration [mg/kg dry tissues] were: for Cu from 13,9 to 21,5; for Zn from 45,8 to 393,1; for Pb from 0,5 to 1,7 and for Cd from 0,1 to 10,8. In kidneys, they were: for Cu from 47,5 to 59,6; for Zn from 381,4 to 415,4; for Pb from 0,1 to 9.2 and for Cd from 6,6 to 37,2 respectively. The obtained results created a baseline for the estimation of current heavy metal accumulation in roe deer in the investigated region. They offer an opportunity that the roe deer in Bulgaria could be used as a bioindicator of future potential anthropogenic negative impact on the environment in forest regions of the country.

INTRODUCTION

The increased environmental pollution by heavy metals led to their accumulation in the wild life (Goyer, 1986). For this reason, the heavy metal pollution can be determined by biological methods with the help of bioindicators – species that can be used as a measure of some aspects of a natural ecosystem's health. Many wildlife species can be used as bioindicators of environmental conditions (Arndt *et al.,* 1987). As accumulative biomonitors of environment pollution, they possess a number of merits such as great availability, low costs, , no servicing, consideration of synergistic (antagonistic) effects, area-related results, biological relevance and comparability to human (Wittig, 1993). The positive features of free-living mammals as bioindicators determined the increased scientific and practical interest for their use in the system of biomonitoring of the natural environment (Wren, 1986; Talmage and Walton 1991; Tataruch and Kierdorf, 2003; Lanocha *et al.,* 2013).

The successful application of roe deer for biomonitoring turned it into one of the main bioindicator species for assessing the accumulation of heavy metals in the wild animal organisms (Wren, 1986; Grodzinska *et al.,* 1983; Tataruch, 1991; Findo *et al.,* 1993; Straub and Kreimes, 1995; Gnamus and Horvat, 1999). Contemporary zoo monitoring studies on the accumulation of heavy metals in the natural environment have proven that the roe deer is a suitable zoomonitor species for this purpose (Tataruch and Kierdorf, 2003) and European roe deer have been used as indicator of environmental contamination for a long time (Sawicka-Kapusta, 1979; Maňkovská *et al.,* 2012).

The aim of this study was to evaluate the concentrations of priority pollutant heavy metal residues – elements with concentration-dependent toxic effect (Cu, Zn) and microelements with proven highly toxic effect on organisms (Cd, Pb) – in target organs (liver and kidneys) of roe deer, originating from a natural population inhabiting Western Stara Planina (Western Bulgaria).

MATERIALS AND METHODS

Study Area

All samples were collected in the region of Svoge municipality, located in Western Bulgaria, in the northern part of South-west region and the northern part of Sofia province (Fig. 1).

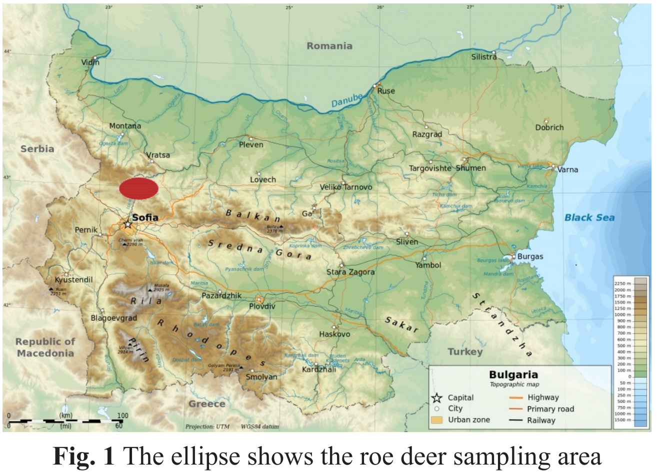

Fig. 1 The ellipse shows the roe deer sampling area

The study area belongs to the western part of the Balkan system morphographic division. The relief is mainly medium and low mountainous, deeply fragmented by Iskar River and its tributaries. The current relief is a complex result of factors with natural and anthropogenic origin, due to which its specific features have been formed – rugged mountainous terrain, steep slopes and deep valley incisions (Team to the Geographical Institute of the Bulgarian Academy of Sciences, 2002). The territorial distribution of the areas by type of land use fully corresponds to the mountain character of the territory – the highest share (59,3%) occupies the lands, intended for forestry purposes. The region is characterized by cold mountain climate, high cloudiness and high relative humidity. The climate regimes on the territory create good conditions for development of forestry and pastoral livestock and only in limited areas – for development of agriculture in mountainous conditions (SFE 'Svoge', 2018).

The choice of this region was predestined by two factors – the hunting specialists determined that the overall environment in the region is favorable to the development of a healthy and viable population of all representatives of hunting fauna, including roe deer. On the other hand, the analysis of the current environmental status and risks on the territory of the Municipality of Svoge has shown that they are of inherent nature and related to the combined impact of natural and anthropogenic factors. The most important among them are atmospheric air pollution from industry, transport, domestic heating, unregulated landfills, construction activities and soil contamination from mining quarries, municipal wastewater, landfill, construction, agriculture and others. (UDMD of Svoge, 2014).

Sample Collection and Processing

The investigated male roe deer were culled during regular hunting period in 2017 in accordance with local hunting plans and regulations and all animals were shot with legal permission. Each animal was characterized by sex and age. All investigated 10 male roe deer inhabited an area covered by forests and arable lands. An experienced hunter, on the base of external characteristics of the shot individuals, estimated their age and subsequently, if necessary, the method of mandible tooth wear (Høye, 2006) was used. Age class was defined as young adults (2-4 years). According to the initial veterinary health inspection, all individuals were in normal physiological condition.

After dissection of the animals in the field, small pieces of liver and kidneys (around 3 g), were immediately removed without external contamination. The tissues were packed separately, frozen and sent to the laboratory, and stored at -20°C until the analyses were performed.

The preliminary preparation of samples included the following steps: a) drying to air dry weight and grinding to a homogenized mass; b) dissolving the sample with a mixture of HNO_3 and $HClO_4$ for 24 h at room temperature; c) evaporating to a wet residue on a sand bath, and d) quantitatively transferring to a test tube and bringing it to a standard final volume with 1N HNO_3.

The content of Pb, Cd, Zn and Cu was determined using an inductively coupled plasma atomic emission spectrometry (ICP-AES) on a Perkin Elmer Optima 7000 DV instrument. Concentrations of elements in the liver and kidney tissues were expressed as mg/kg of dry analyzed tissue.

Statistical analysis

Due to the not too big sample size of our data set, the normality assumption was probably violated. That assumption implied before applying the statistical analysis on the empirical data of metal concentrations in both organs. They were investigated to determine their distribution using the Shapiro-Wilks' W test. It was found that they did not have a normal distribution. For this reason, the reference interval of the residues in both internal organs was calculated using a non-parametrical percentile method. All concentrations were described by their basic descriptive statistics (Mean, Median, Std. Dev.). The range of each variable was determined by the values of 97.5^{th} and 2.50^{th} percentiles – width of the range about the median that includes 95% of the cases. To assess the difference of the observed concentrations of heavy metals studied in both target organs, a nonparametric test (Man-Whitney) for independent samples was applied using the median as a goal and a mean to perform their comparison. Differences were considered as significant at the level of $p<0.05$. The overall metal concentration according to Usero *et al* (1996), $\mathbf{MPI=Cf_1 \times Cf_2 \cdots Cf_n)^{1/n}}$, where Cf_n is the concentration of metal n expressed in the sample) in tissues of both organs was calculated and compared. Statistical analysis of the data was performed using Statistica version 10 (Statsoft Inc., 2011).

To reveal the specificity of heavy metals' residuals obtained in the internal organs of roe deer, they were considered in a comparative aspect with those in the liver and kidney of roe deer, inhabiting the northwestern part of Poland, in an area dominated by agriculture and industry. Forests and forestlands occupy almost 35 % of this area. It is characterized by medium air pollution level. In the northern and eastern part of the province, the pollutant concentrations were low and air pollutants did not exceed permissible levels (Wieczorek-Dąbrowska *et al.,* 2013).

RESULTS AND DISCUSSION

Descriptive statistics for Pb, Cd, Zn and Cu concentration and metal pollution index (MPI) in the liver and kidney are presented in Table 1.

Table 1. Concentrations [mg/kg dry tissue] of Zn, Cu, Pb and Cd in liver and kidney, and metal pollution index (MPI)

Metal	Basic statistical characteristics [mg/kg dry tissues]			Limits of 95% range of the Median [mg/kg dry tissues]		MPI
	Mean	Median	Std. Dev.	From	To	
Liver						10,16
Cu	16,2	14,6	3,5	13,9	21,5	
Zn	150,3	81,3	164,6	45,8	393,1	
Pb	3,5	1,6	0,6	0,5	1,7	
Cd	3,15	0,8	5,1	0,1	10,8	
Kidney						34,51
Cu	52,7	51,1	6,2	47,5	59,6	
Zn	401,5	407,7	17,8	381,4	415,4	
Pb	3,3	0,5	5,1	0,1	9,2	
Cd	20,4	17,5	15,5	6,6	37,2	

Statistical analysis showed that the values of the medians of heavy metal concentrations in both target organs of all investigated elements were specific to each organ. Different rates of accumulation of investigated heavy metals have been detected in the liver and kidneys. We found a great difference between concentrations of Cu and Zn of one hand, and Pb and Cd on the other, in both organs. The rate of Cu and Zn accumulation is about 3 times higher in the kidney. Lead ratio is 1:1, whereas the rate of Cd is around 6 times higher in the kidneys.

The concentrations of the tested metals, specific for each organ, characterized by the width of the range about the median that includes 95% of the cases, demonstrate the ranges of their variability. Typical for the established intervals is that they have wide values in both organs. The higher concentration may resulted from their feeding preferences, digestion rate and feeding frequency. The formal reason for the broad concentration range is that in internal organs of some of the investigated individuals the studied metals were in high concentration.

In our study, we also found that both investigated roe deer organs were characterized by a different metal pollution index. The mean MPI value of the kidney is 3.4 times higher than that of the liver.

The clarification of high difference of the MPI index in both investigated organs needs a further estimation of the concentration of heavy metals in the different components of the ecosystem - leaves, grass, fruit, plant shoots and additional food provided to wild animals in winter.The comparison of the Median's values of heavy metals' concentrations from the current study with those of the roe deer from Poland (in the liver: Cu (12.69 mg/kg dry tissues), Zn (54.92 mg/kg dry tissues), Pb (0.055 mg/kg dry tissue) and Cd (0.770 mg/kg dry tissue); in the kidneys Cu (14.72 mg/kg dry tissues), Zn (81.37mg/kg dry tissues), Pb (0.092 mg/kg dry tissues) and Cd (6.139 mg/kg dry tissues)) showed that their values in the roe deer from Poland were considerably lower than those from Western Bulgaria. At the same time, they fall within the limits of the variability of the investigated heavy metals in the liver and kidney of subadult male roe deer from Western Bulgaria.

CONCLUSION

The obtained results created a baseline for the estimation of current heavy metal accumulation in roe deer in the investigated region and they offer an opportunity that it could be used as a bioindicator of future potential anthropogenic negative impact on the environment in forest regions in Western Bulgaria. Our samples were collected within a small geographical area, characterized by relatively uniform environmental conditions. Some regional differences were found in individual heavy metal element concentrations. In order to identify possible health effects, in relation to trace element toxicities, continued monitoring of tissue concentrations of investigated heavy metals in roe deer is recommended. Our study also reinforces the need to account carefully for specific environmental related variation in ecotoxicological research on wild animals in regions of the country with their own specific ecological conditions.

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CONFLICT OF INTERESTS: The authors have declared that no conflict of interests exists.

AUTHORSHIP STATEMEN: Conceived and created research: G. M., A. A., Acquisition of data: G. M., A. A., Analyzed the data: G. M., A. A., Writing – original draft, review & editing: G. M., A. A.

REFERENCES

1. Arndt, U., Nobel, W., Schweizer, B. 1987. Bioindikatoren: Möglichkeiten, Grenzen und neue Erkenntnisse. Ulmer, Stuttgart, 388 pp. (In German).
2. Findo, S., Hell, P., Farkaš, J., Maňkovská, B., Žilinec, M., Stanovský, M. 1993. Akkumulation von ausgewählten Schwermetallen beim Rot- und Rehwild im zentralen Teil der Westkarpaten (Mittelslowakei). Zeitschrift für Jagdwissenschaft. 39 (3): 181-189. (In German).
3. Gnamuš, A. and Horvat, M. 1999. Mercury in terrestrial food webs of the Idrija mining area. In: Ebinghaus, R., Turner, R. R., de Lacerda, L. D., Vasiliev, O., Salomons, W. (Eds.). Mercury contaminated sites: characterization, risk assessment and remediation. Environmental Science. Springer, Berlin, Heidelberg, pp. 281-320.
4. 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. 3rd ed. Macmillan Publishing Company, New York, pp. 582–635.

5. Grodzinska, K., Grodzinski, W., Zeveloff, S. I. 1983. Contamination of roe deer forage in a polluted forest of southern Poland. *Environmental Pollution,* Series A. 30: 257-276.
6. Høye, T. T. 2006. Age determination in roe deer – a new approach to tooth wear evaluated on known age individuals. *Acta Theriologoca.* 51 (2): 205-214.
7. Lanocha, N., Kalisinska, E., Kosik-Bogacka, D. I., Budis, H., Sokolowski, S., Bohatyrewicz, A. 2013. Comparison of metal concentrations in bones of long-living mammals. *Biological Trace Element Research.* 152 (2): 195-203. doi:10.1007/s12011-013-9615-x
8. Maňkovská, B., Oszlányi, J., Goryanova, Z. I., Frontasyeva, M. V., Kastier, P. 2012. Regional variation in environmental element concentrations in Slovakia derived from analysis of roe deer teeth (Capreolus capreolus L.). *Ekológia* (Bratislava). 31 (2): 138-149.
9. Sawicka-Kapusta, K. 1979. Roe deer antlers as bioindicators of environmental pollution in southern Poland. *Environmental Pollution.* 19 (4): 283-293.
10. SFE "Svoge". 2018: Site of State forestry „Svoge": www.dgs-svoge.szdp.bg
11. StatSoft, Inc. STATISTICA (data analysis software system), version 10, 2011. www.statsoft.com
12. Straub, H. R and Kreimes, K. 1995. Rehe als Akkumulation-sindikatoren. In: Umlauff-Zimmermann, R. (Ed.). Methoden zu Wirkungserhebungen. Landensanstalt fur Umweltschultz, *Karlsruhe*, pp. 52-54. (In German).
13. Talmage, S. and Walton, B. 1991. Small mammals as monitors of environmental contaminants. Review of Environmental Contamination and Toxicology, 119: 47-108.
14. Tataruch, F. 1991. Freilebende Wildtiere als Bioindikatoren der Schwermetallkontamination. VDI Berichte. 901: 925-936. (In German).
15. Tataruch, F. and 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. Elsevier, Amsterdam, pp. 737–772.
16. Team to the Geographical Institute of the Bulgarian Academy of Sciences, 2002. Geography of Bulgaria: physical and human geography. ForCom Publishing. pp. 760 (In Bulgarian).
17. UDMD of Svoge. 2014: A updated document for the implementation of the Municipal development plan for the municipality of Svoge in the period 2014-2020. http://www.svoge.bg/wp-content/uploads/2012/02/PlanZaRazvitieNaObshtinaSvoge.pdf
18. Usero, J., Gonzalez-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.
19. Wieczorek-Dąbrowska, M., Tomza-Marciniak, A., Pilarczyk, B., Balicka-Ramisz, A. 2013. Roe and red deer as bioindicators of heavy metals contamination in north-western Poland. *Chemistry and Ecology.* 29 (2): 100-110.
20. Wittig, 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.
21. Wren, D. 1986. Mammals as biological monitors of environmental metal levels. *Environmental Monitoring and Assessment,* 6 (2): 127–144.