European Roe Deer (*Capreolus capreolus*) as a Biomonitor for Contemporary Heavy Metal Pollution of the Environment in Forest Mountain Regions in Rhodope Mountains, Bulgaria

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Abstract. The aim of the study was to check the current loaded with heavy metals (Pb, Cd, Cu and Zn) liver and kidney tissues of European roe deer (*Capreolus capreolus*) inhabiting typical forest mountain region in Bulgaria. The roe deer's liver and kidneys were used as test systems for determination of analytic concentrations of the studied heavy metals by an inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Perkin Elmer Optima 7000 DV. The concentrations [mg/kg dry tissues] of the tested metals, specific for each organ showed the limits of their variability. In the liver, the limits of the variability of metal concentration were as follows: for Cu from 29.65 to 348.86; for Zn from 13.6 to 166.57; for Pb from 0.75 to 4.78 and for Cd from 0.025 to 3.22. Respectively in the kidneys, they are for Cu from 35.66 to 61.70; for Zn from 81.41 to 224.47; for Pb from 0.35 to 9.22 and for Cd from 8.87 to 35.82. The results of this study show that the roe deer in the mountainous regions of the Rhodope Mountains is suitable for use as a biomonitor for contemporary heavy metal environmental pollution in the trophic area of the big game in the forest mountain regions of Bulgaria. In addition, the established results could be used to create a baseline for the estimation of current heavy metal accumulation in roe deer. They also offer an opportunity for it to 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: roe deer, *Capreolus capreolus*, heavy metals, forest mountain ecosystems, environmental pollution.

Introduction

The European roe deer (*Capreolus capreolus* Linnaeus, 1758) is common for Bulgaria and is a typical game species in the terrestrial ecosystems of the country. The potential inhabitable territory in the country is about 7.55 million hectares, of which 3.77 million hectares are field resources and include biotopes with various ecological conditions. The survey of the game in Bulgaria conducted in the spring of 2018 indicates that there are about 116,000 roe deer in the country (DZHUPAROV, 2018). In Bulgaria, as in other European countries, it is one of the key species of European terrestrial ecosystems (ANDERSEN *et al.*, 1998; APOLLONIO *et al.*, 2010) and so, it is of great importance in forest mountain area.

The main threats to wildlife health from heavy metals are associated with exposure to

non-essential elements, such as lead (Pb) and cadmium (Cd). The anthropogenic pollution of ecosystems with essential heavy metals (Zn and Cu) is also problematic at some sites. These latter two metals can be toxic to wildlife if the concentration of available metal in it is high enough (ENVIRONMENTAL FACT SHEET, 2005). 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).

Therefore, it is necessary and important to check heavy metals concentrations in the environment, in areas that are not directly related to pollution sources in forest mountain regions.

Wild animals, especially game species, such as roe deer, are suitable as biomonitor (FROSLIE *et al.*, 2001) due to their large geographical distribution, a residential way of life, feeding habits, relatively long lifespan and easy sample collection (regular hunting activities). For sustainable use of the potential of roe deer as a natural resource and for balanced development of terrestrial ecosystems, which it inhabits, it is crucial to assess the degree of load of its internal organs (liver and kidneys) with toxic elements (TATARUCH & KIERDORF, 2003)

The aim of the study was to measure the concentrations of several toxic elements (Cd, Cu, Pb and Zn) in the liver and kidney tissues of roe deer inhabiting typical forest mountain area in the Rhodope Mountains, Bulgaria. The established in the current survey reference values of their residuals in roe deer from forest mountain trophic areas would give an opportunity to use this species as a biomonitor of future potential anthropogenic negative influence on the environment in forest mountain area in the country, under the conditions of modern industrial activities in Bulgaria.

To reveal the specificity of heavy metals' residuals obtained in the internal organs of roe deer inhabiting the landscape in Rhodope Mountains, Bulgaria they are considered in a comparative aspect with to 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 a medium air pollution level. In the northern and eastern part of this region, the pollutant concentrations of heavy metals are low and permissible levels of air pollutants are not exceeded (WIECZOREK-DĄBROWSKA *et al.*, 2013).

Materials and Methods

Field study

The roe deer included in the present study are 15 adult male roe deer, between 2 and 5 years. They were legally hunted from different areas in forest mountain regions in the Rhodope Mountains during the hunting season of 2017 in southern Bulgaria. The age of the roe deer was determined by analyzing their exterior features and the degree of development and wear of their teeth (HOYE, 2006).

The study area represents a forest mountain region, which is a typical trophic area of the big game in the Rhodope Mountains (Fig. 1), where woody vegetation is varied and up to about, 1200 m altitude is formed of oak and beech formations. The natural features of the studied territory, such as diversity of terrain, wood, shrub and grass vegetation, as well as the abundance of spring and river water, favor the presence of a number of hunting economic mammal species, such as red deer, fallow deer, mouflon, roe deer and wild boar.

Chemical analyses

All investigated individuals of roe deer according to the initial veterinary health inspection were in normal physiological condition. The livers and the kidneys were used as test systems for the determination of analytic concentrations of the studied heavy metals (Pb, Cd, Cu and Zn) in the roe deer specimens. A small piece of liver and kidney

tissues, around 3 g, without external contamination was removed from each roe deer immediately after its shooting. 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 to establish the analytical concentration of the investigated heavy metals, by methods of atomic spectrometry, includes 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 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 analyzed samples was determined using an inductively coupled plasma atomic emission spectrometry (ICP-AES) in a Perkin Elmer Optima 7000 DV instrument, based in the Chemical Laboratory of the Institute of Biodiversity and Ecosystem Research at Bulgarian Academy of Sciences, Sofia The residual amounts of the studied elements were established using an atomic-absorption analysis and expressed as mg/kg of dry analyzed tissue.

Statistical analyses

The reference interval of the residues of all investigated heavy metals in both internal organs was calculated using a non-parametrical percentile method. All concentrations were described by their basic descriptive statistics (Median; Std. Dev. and 2. 50 and 97. 5 percentile value). 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 assess the difference of the observed concentrations of the studied heavy metals in both target organs, a nonparametric test (Man-Whitney test) for independent samples was applied using the median as a goal and a means to do their comparison; the p-value of 0.05 was treated as a "border-line acceptable" error level. All calculations were performed using the statistical package STATISTICA, version 10.0 (STATSOFT INC., 2011).

Fig. 1. The black point marks the location of the roe deer sampling area in the Rhodope Mountains in Southern Bulgaria.

Results and Discussion

Descriptive statistics for residuals of Cu, Zn, Pb and Cd in the liver and kidney tissue of the investigated roe deer are presented in Fig. 2.

The results of the Man-Whitney test showed that the differences of the medians of the observed heavy metals' concentrations in both target organs were of different statistical significance: for Cu and Pb it is $p > 0.05$, while for Zn and Cd it is $p < 0.05$. As could be seen, the measured concentrations of Zn and Cd were specific to each one of the target organs, while the median value of the concentrations of Cu and Pb do not differ at p-value, which was treated as a "borderline acceptable" error level.

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 show the limits of their variability (Table 1).

The overview of the Median's values of the concentrations of the heavy metals resulting in the current study with those of the roe deer from Poland (WIECZOREK-DĄBROWSKA *et al.*, 2013) 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)) shows that their values in the roe deer from Poland are considerably lower than those in the roe deer inhabiting in Rhodope Mountains. At the same time, they do not fall (with the exception of Cu concentration in the liver) within the limits of the variability of the investigated heavy metals in the liver and kidney of adult male roe deer from of the environment in forest mountain regions in Rhodope Mountains, Bulgaria.

Relatively broad limits of the studied heavy metals variability are predetermined by the high load on the internal organs of individuals with some of these metals. This effect is probably related to the heterogeneity of the load on the separate components of the environment in the Rhodope Mountains both because of natural geochemical characteristics and because of human activities, which greatly impair the natural environment in many parts of the mountain. 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).

The presence of Pb and Cd with proven highly toxic effect on living organisms in considerable concentrations, together with the noteworthy presence of elements with dependent toxic effects elements (Cu & Zn) in sizeable residues in the internal organs of some roe deer, as well as the potential for deterioration of the quality of the environment in the mountainous habitats of the roe deer in the Rhodope Mountains, as a result of anthropogenic activity, 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.

Table 1. The limits of variability of the concentrations [mg/kg dry tissues] of the investigated heavy metals in liver and kidney of adult male roe deer inhabiting environment in forest mountain regions in Rhodope Mountains, Bulgaria.

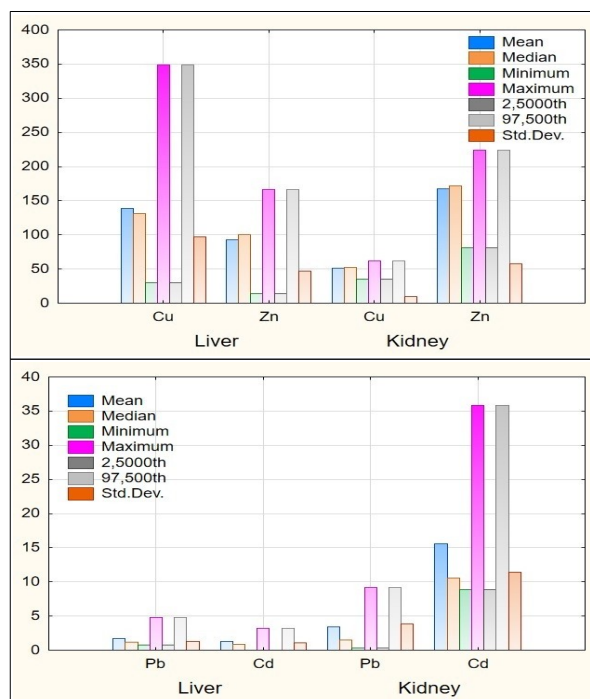|	Liver							
Metal	**Cu**		**Zn**		**Pb**		**Cd**	
Limits	From	To	From	To	From	To	From	To
	29.65	348.86	13.67	166.57	0.75	4.78	0.025	3.22
	Kidney							
Metal	**Cu**		**Zn**		**Pb**		**Cd**	
Limits	From	To	From	To	From	To	From	To
	35.66	61.70	81.41	224.47	0.35	9.22	8.876	35.82

Fig. 2. Values of basic statistical characteristics of the concentrations [mg/kg dry tissues] of the heavy metals Cu and Zn, (A), and Pb and Cd (B) in livers and kidney of adult male roe deer inhabiting Rhodope Mountains (Southern Bulgaria).

Conclusions

The results of this study show that the roe deer in the mountainous regions of the Rhodope Mountains is suitable for use as a biomonitor of contemporary heavy metal environmental pollution in the trophic area of the big game in the forest mountain regions of Bulgaria. The established results describing heavy metal content in its internal organs create a baseline for the estimation of current heavy metal accumulation in roe deer and offer an opportunity for it to be used as a bioimonitor of future potential anthropogenic negative impact on the environment in forest regions of the country, under the conditions of modern anthropogenic activities therein.

Acknowledgements

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