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Assessing the Heavy Metal Content in Forest Dormouse (Dryomys nitedula Pallas, 1778) from an Agricultural Region in Bulgaria

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Abstract. The heavy metals load in the forest dormouse (*Dryomys nitedula*), inhabiting in forest shelter belts in the agricultural region was assessed. The concentrations of Cd, Co, Cu, Ni, Pb and Zn (expressed in mg/kg of dry tissue) were established in the liver, using an atomic-absorption analysis. The fact that the highly toxic metals (Cd and Pb) were found in considerable concentrations together with other metals with concentration dependent toxic effect (Cu, Ni, Zn and Co) in the liver of forest dormice, suggests that it is necessary to carry out regular assessment and forecasting of accumulation of these metals in species, which are not direct targets of cultivation and control activities in agricultural ecosystems. The obtained values were used to create a baseline for estimation of heavy metal accumulation in the internal organs of the forest dormouse, both in anthropogenically transformed habitats and natural biotopes, as well as for using this species as a monitor of environmental status.

Key words: heavy metals, forest dormouse, forest shelterbelts, *Dryomys nitedula.*

Introduction

Due to the typical for the species high degree of ecological adaptation (AIRAPETYANTZ, 1983) the forest dormouse (*Dryomys nitedula* Pallas, 1778) is able to inhabit biotopes in the forest ecosystems throughout much of Eurasia (BATSAIKHAN *et al.*, 2008). Its area covers the territory of Europe from the Alps southward, including the Balkan Peninsula, northward to the Baltic Sea, and eastward to Volga and the Ural Mountains. The distribution of the forest dormouse inside the area is sporadic (KRYŠTUFEK, 1999).

In Bulgaria, as well as in the biggest part of its European area (KRYŠTUFEK, 1999) the forest dormouse occurs mainly in deciduous and coniferous woodlands, wherever suitable conditions are present. The presence of bushes and thick undergrowth are the main habitat requirements, determining the present distribution of this species in Bulgaria too (MARKOV, 1959). Under these conditions the forest dormouse is able to inhabit biotopes in many natural mountainous and hilly forest ecosystems throughout Bulgaria (MARKOV, 2003), which are quite variable in

their physical geographical conditions (TISHKOV, 1976). The forest dormouse usually avoids human dominated habitats such as agricultural areas (BATSAIKHAN *et al.*, 2008).

During the second half of the 20th century in the fields of North-eastern Bulgaria artificial forest plantations of basic and concomitant vegetation (trees and bushes), forming the forest shelter belts have been created. These artificial forest plantations have created new, potentially suitable favourable biotopes for the forest dormouse in Bulgaria. They provide various plots of deciduous forest with shrubby layer and dense undergrowth, which are the most optimum stations in the forest dormouse habitats. Nowadays the forest dormouse is settled permanently there and develops vital populations. The newly established populations of the forest dormouse in the agricultural regions of the country exist under ecological conditions that are influenced by the surrounding anthropogenic agricultural activities (MARKOV *et al.*, 2009).

Given the growing human impact on the biosphere, the heavy metals represent a special danger for the agricultural biocoenoses. Getting into the soil and plants they accumulate in the agricultural ecosystems and get involved in the metabolic cycles of living organisms; forming highly toxic carcinogenic compounds and causing negative reactions, they create unfavorable conditions for the existence of the living organisms (WHO, 1992).

Because of the ability of wild mammals to accumulate heavy metals in their internal organs such as liver and kidney (GOYER, 1986), they have been recognized as valuable biological monitors of xenobiotic pollutants present in the ecosystem (VENOGOPAL & LUCKEY, 1978; WREN, 1986; TALMAGE & WALTON, 1991). The evaluation of accumulating contaminants of heavy metals levels, which belong to the group of the most dangerous inorganic toxic substances (LUCKEY *et al.*, 1974) in wild animals is important not only for assessing the potential effects of pollutants on their health status, but also for obtaining information about the quality of the ecosystem, an essential part of which are the animals (TATARUCH & KIERDORF, 2003).

Forest dormice are omnivores and their diverse food spectrum includes both animal foods, such as birds, eggs, insects, terrestrial non-insect arthropods, and plant foods: leaves, seeds, grains, nuts and fruit (AIRAPETYANTS, 1983; LOZAN, 1970). They have small home ranges (about 65–100 m long), strongly associated with tree and shrub habitats, good reproductive potential (one female gives birth to 2-9 cubs each year, most often 4-5 cubs), relatively short lifespan (3-5 years in wild, more often 4 years) (BATSAIKHAN *et al.*, 2008; ŚCIŃSKI & BOROWSKI, 2006; LOZAN, 1970; 1979; NOWAKOWSKI, 2001; SLUDSKII, 1977; AIRAPETYANTS, 1983). These biological features together with the fact that it is widely spread in Bulgaria (MARKOV, 1959; 2001) and Europe (KRYŠTUFEK, 1999) make the forest dormouse an useful species for the risk assessment of heavy metals accumulation in the wild animals and environmental monitoring.

The lack of knowledge about the actual values of the concentrations of heavy metals in natural populations of forest dormice prompted the present investigation. The aim was to evaluate the concentrations of residues of priority pollutants of the heavy metal group (Cu, Ni, Zn, Co, Pb and Cd) in samples from the target organ (liver) of forest dormice, inhabiting the main agricultural region of North-eastern Bulgaria and to provide a basis for their future monitoring in natural and agricultural ecosystems in the Eurasian range of the species.

To assess the specificity of heavy metal bioavailability in the forest dormouse, the heavy metals residuals in the liver samples were compared with the residuals in the liver of specimens from two other species: one related – the fat dormouse (*Glis glis*) and one differing by biological and ecological characteristics – the common vole (*Microtus arvalis*). The data were obtained during previous studies on heavy metal levels in small mammals in the same agricultural

region (MARKOV, 2012). Both dormice species are sympatric and occupy permanently the tree-shrub layer in the artificial forest shelterbelts. They differ by their lifespan – shorter in the forest dormouse (GOLODUŠKO & PADUTOV, 1961; PILASTRO *et al*., 2003) and diet – the animal component is more strongly represented in the food spectrum of the forest dormouse (NOWAKOWSKI & GODLEWSKA, 2006). The common vole lives underground in colonies across open agricultural lands, which are intensively treated with different chemicals for increasing crop yields. This species is territorially conservative. The average lifespan of the common vole is too short – about 4.5 months. The mean litter size is close to 5 cubs. The common vole is a typical herbivorous rodent with a food spectrum including more than 80 plant species and preferences to cereals, *Asteraceae* and *Fabaceae* (SOKOLOV & BASHENINA, 1994).

Material and Methods

Specimens of the forest dormouse were obtained from forest shelterbelts during a study of the presence and abundance of small mammal pests (*Microtus* spp., *Apodemus* spp. and *Mus* spp.) in an agricultural region in the Shumen District of North-eastern Bulgaria. The study plot covered an alfalfa field, the adjacent corn field and forest shelterbelt, as well as the nearby undeveloped area of the primary steppe biotope.

To avoid the potential influence of the specimens' age and gender on the quantitative accumulation of heavy metals in their bodies, only adult males (> 3 years) was analysed. Age determination of the forest dormouse was based on the degree of the wear of tooth enamel (LOZAN, 1961); gender was determined by external appearance and confirmed by dissection.

In the liver samples from 10 adult males forest dormouse the residual amounts of the studied elements (Cd, Co, Cu, Ni, Pb and Zn) were established using an atomic-absorption analysis. The heavy metal concentrations were determined with a Perkin-Elmer Model 3030B atomic absorption spectrophotometer with an air/acetylene flame, and expressed as mg/kg of dry analysed tissue. Before analysis, all samples were dried to constant weight at 60 ºC (HAVEZOV & TSALEV, 1980). The homogenization of each sample was performed by crushing in a porcelain mortar. Subsamples of 1 g were removed and transfer to an iodination flask. There it was wetted with distilled water and a 15 ml of a concentrated $HClO_4$ and HNO_3 acids were added. After the sample stayed for 24 hours at room temperature, it was heated in a sand bath to wet residue. The solutions were made up to 10 ml with 1n HNO_3. The digestion of samples was carried out in duplicate to ensure the reproducibility of the method. Analytical grade reagents were used to make up the relevant blanks and calibration curves

The basic statistical parameters: the mean (X), the standard deviation (SD), the standard error of the mean (SE) and the ± 95% confidence interval of the mean values were calculated for each investigated residual concentration of heavy metals in the liver of forest dormouse**.**

The significance of differences between the residuals found in the forest dormouse liver during the present investigation and the data for the fat dormouse and common vole obtained by MARKOV (2012) was tested in the Mann - Whitney U-test. The difference is insignificant since $p>0.05$

All calculations were performed using the statistical package STATISTICA version 8.0 (STATSOFT INC., 2008).

Results

The mean values of the residual heavy metals (Cd, Pb, Ni, Zn and Cu) found in the liver of the adult male forest dormice inhabiting forest shelter belts in the main agricultural region of North-eastern Bulgaria and their statistical estimation are presented in Table 1.

According to the empirically obtained mean values of the heavy metals residuals the elements studied in the liver could be arranged as follows: Zn > Cu > Pb > Ni > Co > Cd. This sequence of the heavy metals residuals in the liver of the forest dormouse corresponds to the relative concentrations of

these metals found in rodents inhabiting non-loaded or extremely loaded environments or obtained during experimental investigations under laboratory conditions (ŠUMBERA *et al.*, 2003).

Table 1. Heavy metals residuals X [mg/kg dry weight], their standard deviation (SD) and ±95 % confidence limits (C. L.) in liver of forest dormice (*Dryomys nitedula*) from the forest shelterbelt in an agricultural region in North-eastern Bulgaria

Metal	Mean	S.D.	-95 % C. L.	+95 % C. L.
Zn	63,102	6,833	46,128	80,075
Cu	12,387	1,536	-	16,202
Ni	0,533	0,145	0,174	0,892
Co	0,529	0,121	0,230	0,828
Cd	0,310	0,153	-	0,690
Pb	2,381	0,525	1,078	3,685

The highest absolute variation (SD) was found in the mean values of Zn and Cu residuals in the liver. The absolute variation of the mean value of Pb was also high. The absolute variation of the mean values of the remaining three elements – Ni, Co and Cd was of similar magnitude. The mean values of Cd, Ni, Co and Pb showed high relative variation with the highest rate in Cd – almost 50%. In Zn and Cu it was about 10%. The mean values of the residuals of all metals were obtained with a relatively low rate of the arithmetic mean: from 29 % in Cd to 6% in Zn.

The bioavailability comparison of the studied heavy metals in the forest dormouse liver with those in the fat dormouse liver and the common vole liver (Fig. 1) showed:

(i) The mean values of the Zn, Cu, Pb, Ni and Co concentrations in both dormice species were similar and the differences between them were statistically insignificant. The mean concentration of Cd in the forest dormouse was higher than the concentration found in the liver of the fat dormouse, where it was less than <0.001 mg/kg. Probably the absence of statistical significance in the empirical differences in the mean values of the heavy metals residuals in both closely related dormouse species was due to the similar habitat structures they were using (shrubby layer) in the forest shelterbelts, i.e. they lived under equal ecological conditions. This habitat was not directly used for farming and the dormice were non-target species for anthropogenic activities there.

(ii) The empirically found mean values of the residuals of the studied heavy metals in the liver of the common vole were higher than in the forest dormouse, but still statistically insignificant. Because of the high individual variation of the studied metals concentrations in the liver of the common vole, the mean values of the residuals found in the liver of the forest dormouse fall within their wide bounds and the 95% confidence intervals of their mean values in both species overlapped.

The common vole is a target species of human activities associated with the usage of xenobiotics in soil treatment, aiming to reduce pest numbers in agricultural areas. It is also directly affected by tillage. Probably, the different level of impact of these two anthropogenic factors determines the degree (from low to extremely high), in which substances, imported into the agricultural ecosystem and containing heavy metals, affect the common vole individuals.

Discussion

Toxic metals are ubiquitous in the environment (POHL *et al.*, 2011). Because of human activities, animals can be exposed to abnormal amounts of toxic metals. This exposure can lead to the accumulation of pharmacologically significant concentrations of metals in animal tissues.

The present work gives a warning about the bioavailability of heavy metals in forest dormouse in the country's plain regions. For the first time in this study initial norms of their variation in typical agricultural landscapes are given. The found concentrations of priority pollutant residues from the metal group – elements with a concentration dependant toxic effect (Cu, Ni, Zn, Co) and microelements with a proven highly toxic effect on living organisms (Cd, Pb) provide information on the actual problems

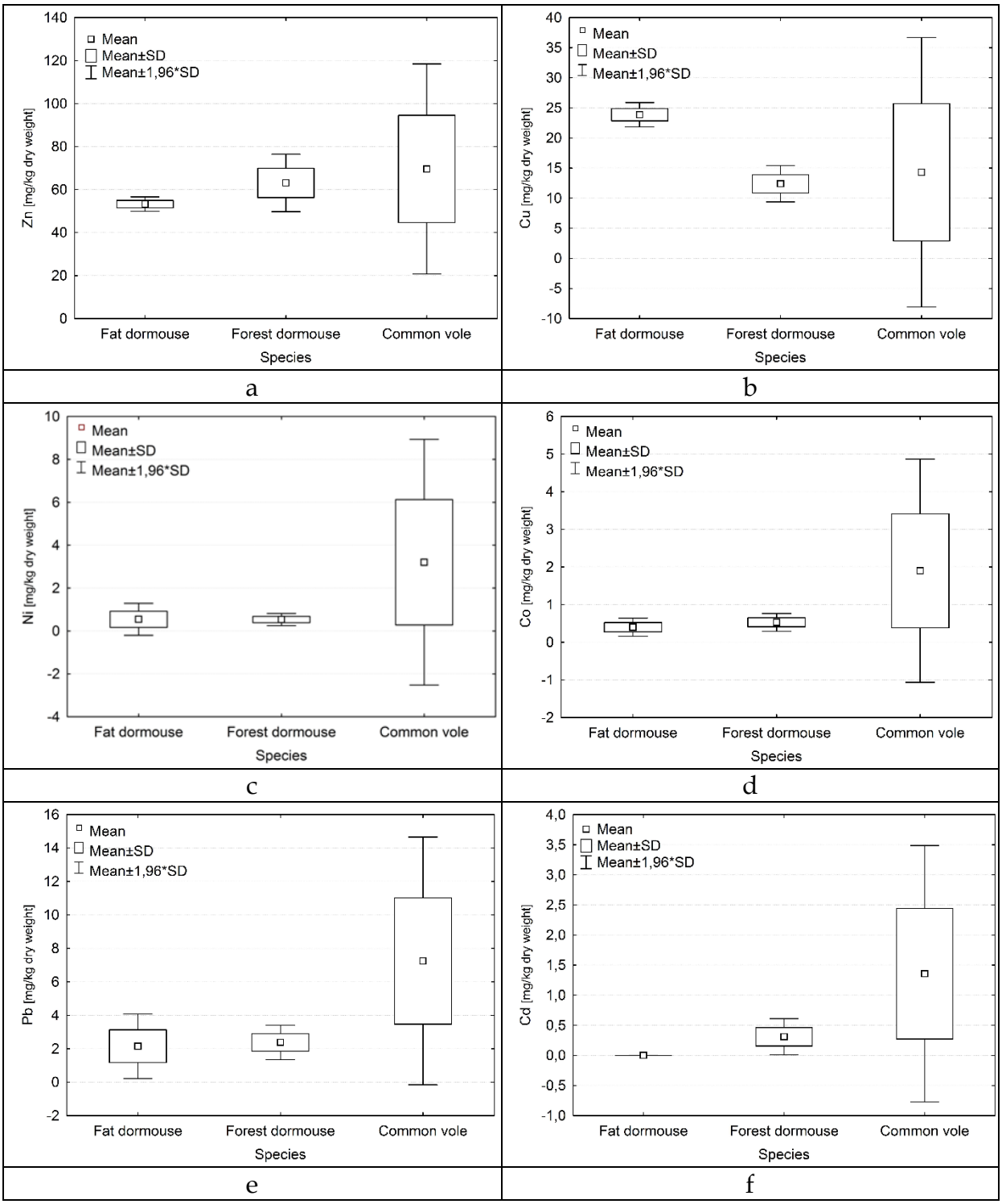

Fig. 1. Heavy metals residuals Mean [mg/kg dry weight and their statistical evaluation in liver samples of forest dormouse (*Dryomys nitedula*), fat dormouse (*Glis glis*) and common vole (*Microtus arvalis*) from the forest shelterbelt in North-eastern Bulgaria. Combined results of this study (liver form forest dormouse) and the reference data for fat dormouse and common vole (after MARKOV, 2012).

associated with potentially increasing anthropogenic pollution of the environment and the quality of the agricultural ecosystems of which the forest dormouse is an intrinsic part. They show that highly toxic elements, such as lead and cadmium

(KABATA-PENDIDAS & PENDIDAS, 1979; LUCY & VENUGOPAL, 1986), could be found in considerable concentrations in the liver of forest dormouse. The increased presence of these elements in the bodies of wild animals is commonly accepted because of the anthropogenic pollution of the environment (SAWICKA-KAPUSTA, 1979).

If the concentrations of essential elements, such as Cu, Ni, Zn, Co, are increased, they could turn into toxic agents when ingested in excess (CLEMENS, 2006; SINGH *et al*., 2011), and their accumulation should be traced in pollution bio-monitoring and environment hazard assessment.

Conclusions

As the residuals of microelements with a proven highly toxic effect and essential elements with a possible toxic effect (when their normal physiological concentration is exceeded) were found in the forest dormouse, their accumulation in the bodies of these animals should be tracked out as a component of the biomonitoring of agricultural ecosystems. Clearly, the survey of the heavy metals residues in the forest dormouse tissues, together with finding out of their harmless levels in future should aid in expanding our knowledge of the anthropogenic impact on agricultural lands, because this species represents an intermediate stage between low and high trophic levels in the ecosystems, where it inhabits.

The development of this task could provide new opportunities for using this species as a monitor of environmental status, both in new anthropogenically transformed habitats and in natural biotopes over its European range. A regular monitoring of heavy metal burdens in forest dormouse, together with monitoring of various physical, chemical and biological components of an ecosystem will provide important data regarding the bioavailability of contaminants within natural ecosystems and the health status of the species.

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