Oecologia Montana 2018,
27, 27-31

Howell-Jolly bodies in red blood cells of snow vole *Chionomys nivalis*

M. BIELESCH and M. JANIGA

Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic; e-mail: marekbielesch@gmail.com; janiga@uniza.sk

Abstract. This study focused on counting Howell-Jolly bodies in peripheral blood of *Chionomys nivalis* and *Myodes glareolus* within the study area (High Tatras - Biele plesá). The number of Howell-Jolly bodies was similar in both species and did not differ between males and females or adults and non-adults. Only five samples contained relatively more Howell-Jolly bodies (4 samples contained four and one sample contained five Howell-Jolly bodies per 1000 erythrocytes). We also compared the number of Howell-Jolly bodies to the amount of heavy metals found in the vertebrae of snow voles. The quantity of Howell-Jolly bodies in peripheral blood of animals in relation to the amount of lead in bones was found to be statistically insignificant but we found an increased number of Howell-Jolly bodies in relation to on the concentration of molybdenum.

Key words: Chionomys nivalis, Myodes glareolus, hematology, Howell-Jolly bodies, High Tatras, pollution

Introduction

Pollution represents a critical problem, particularly in heterogeneous landscapes, such as mountainous regions, where we observe a higher impact than in low lying areas. Recent studies demonstrable clearly that high alpine environments are negatively influenced by environmental pollution. Atmospheric inputs at high elevations are greater than those oflow elevation regions, because of orographic effects, cloud deposition and wind speed (Lovett and Kinsman 1990). Although adverse health effects of heavy metals have been known for some time, exposure to heavy metals continues, and has even increased in some parts of the world, particularly in less developed countries (Jarup 2003). The presence of metals in the environment always represents a risk for living organisms, and the effects of heavy metals may lead to morphological, physiological, pathological and genetic changes. The most common pathological changes affect the blood and organs(Jančová *et al.* 2004). According to Rostal *et al.* (2012), hematological data are important indicators out of the condition of wild animal populations affected by pollutants. One of the main hematological parameters used to determine the effects of heavy metals in small mammals is the presence of Howell-Jolly bodies in erythrocytes. Erythrocytes are blood cells, which transport oxygen to the body's tissues through the circulatory system. Howell-Jolly bodies were described as micronuclei or "fragment of nuclear material" in the cytoplasm of erythrocytes by Howell and Jolly in the late 1800s and early 1900s. The first innovators, Evans *et al.* (1959) discovered the applicability of micronuclei as markers for cytogenetic damage. Several researchers have recommended the use of micronuclei as a biomarker in testing on counting micronuclei in the erythrocytes of small mammals. Significant correlation between heavy metal contamination and presence of Howell-Jolly bodies in erythrocytes has been detected in rodents living near polluted areas (Tapisso *et al.* 2009).

In some studies, Snow voles caught in the spring exhibited significantly higher micronuclei frequencies in peripheral blood than individuals trapped in summer or winter. This result correlates with other studies in small mammals, reporting an association of micronuclei frequencies and metal concentrations (Ieradi *et al.* 1996, Topashka-Ancheva *et al.* 2003, Metcheva *et al.* 2008).

The main goal of this study was to observe the Howell-Jolly bodies in the peripheral blood of *Chionomys nivalis* in the Tatra mountains. The number of Howell-Jolly bodies in *Chiononyms nivalis* was compared to the number of Howell-Jolly bodies in the blood of *Myodes glareolus* to examine the relationship lead and molybdenum concentrations in the tail vertebrae and the number of Howell-Jolly bodies in the blood.

Material and Methods

Local populations of snow voles in the Dolina Bielych plies valley region of the High Tatras were sampled as part of this study. *Chionomys nivalis* and *Myodes glareolus* were regularly sampled from June to November 2016. The study area included alpine meadows, with typical geological, plant, and moss features for this type of ecosystem.

Live traps were distributed in the field approximately 5 to 10 m apart from one another. Approximately 80 traps were checked per day. The fieldwork took place over 18 days. Coordinates were recorded with GPS to mark the location of trapped

individuals. Photo and video samples were taken for use in additional evaluation of plants, mosses, and geological features of the study area.

Sherman traps were baited with fresh apples, seeds, cereals and peanut butter. Dry grass was also added to the traps as bedding material, mainly during cold days. Each of the captured individuals was identified. Part of the tail (app. 0.03 cm) was clipped from each captured animal, after animals were anaesthetized with Isoflurane. Peripheral blood was collected from the tail vena (*vena caudalis*).

Two slides were prepared for each individual. A drop of blood was smeared on each slide to make a thin smear. The blood smears were air-dried and placed in a slide box. Following measurement, the blood smears were stained according to Doubek *et al.* (2003).

Stained blood smears were numerically evaluated with the use of immersion oil under 1 000 times magnification using a meandering movement of the slides. For each individual, micronucleus frequency was scored on 1 000 erythrocytes.

The sex was identified through location of genitals. Genitals of female rodents are situated closer than male genitals. In sub-adult animals, there are distinguishable small nipples in females and abdominal testes in males (Balčiauskienė *et al.* 2009).

We also recorded the date individuals were trapped, body length, tail length and hind foot length of individuals. Weight was taken using a spring scale (Pesola 100 g). After these measurements animals were immediately released at the point of capture.

The ED-XRF spectrometer delta was used to determine chemical composition, mainly the amount of heavy metals (Pb, Mo, and etc.) in bones from the small part off the tail.

Animals were categorized into three groups according to the number of Howell-Jolly bodies present. Individuals from the first group had less than 2 Howell-Jolly bodies per 1 000 erythrocytes (Category 1), the second group presented with 2 Howell-Jolly bodies per 1 000 erythrocytes (Category 2) and the third group with more than 2 Howell-Jolly bodies per 1 000 erythrocytes (Category 3). This data was input from Microsoft Excel to STATISTICS 12.1, which was used for statistical analysis. The data was standardized and one-way analysis of variance (ANOVA) was used to determine any significant differences between two or more independent groups of parameters. The data are expressed as mean ± standard error. P-values less than 0.05 were considered to be statistically significant and values more than 0.05 were considered to be statistically insignificant.

Results

In this study we evaluated peripheral blood samples of *Chionomys nivalis* and *Myodes glareolus*. A total of 95 samples were taken in the High Tatras during 2016. The main objective was to count the number of Howell-Jolly bodies per 1 000 erythrocytes in blood smears taken from peripheral blood of *Chionomys nivalis*. The animals were classified into three groups. They were based on the number of Howell-Jolly bodies found in peripheral blood:

- Category 1: less than 2 Howell-Jolly bodies per 1000 erythrocytes
- Category 2: 2 Howell-Jolly bodies per 1 000 erythrocytes
- Category 3: more than 2 Howell-Jolly bodies per 1 000 erythrocytes

It was found that hematological parameters are not significantly related to the number of Howell-Jolly bodies found in the blood. *Myodes glareolus* lives in this habitat at the upper limit and can be more sensitive than *Chionomys nivalis*, which has an upper limit at higher altitudes. It appears that habitat is a factor in higher numbers of Howell-Jolly bodies in erythrocytes of *Myodes glarelous*. Ratios of males to females in both species did not vary over the three categories based on the number of Howell-Jolly bodies (Table 1 and 3). Ratios of adults and non-adults were also unchanged in all three categories based on the amount of Howell-Jolly bodies (Table 2 and 4).

Chionomys nivalis individuals with higher amounts of lead in the tail vertebrae tended to have more Howell-Jolly bodies, though differences among the three Howell-Jolly categories were not statistically significantly different (Fig. 1). Ratios of males and females did not vary in the three categories based on the amount of Howell-Jolly bodies (Table 1). Ratios of adults and non-adults were not different in the three categories based on amount of Howell-Jolly bodies too (Table 2).

Animals with more Howell-Jolly bodies tended to have statistically significant increased levels of molybdenum in their tail vertebrae (Fig. 2). Ratios of males and female were not different between the three categories, based on the amount of Howell-Jolly bodies (Table 1). Ratios of adults and non-adults were not different between the three categories based on amount of Howell-Jolly bodies too (Table 2).

Discussion

Chionomys nivalis has been used as a bioindicator in environmental studies performed in Bulgaria, Spain, Czech Republic, Italy, Slovakia and others (Ieradi *et al.* 1996, Topashka-Ancheva *et al.* 2003, Metcheva *et al.* 2008, Janiga *et al.* 2012).

Çavuoglu *et al.* (2010) observed the number of Howell-Jolly bodies in the blood of animals and described inclusion as an important indicator to detect genetic damage induced by chemicals or radiation. The inclusion test showed a significantly higher frequency of Howell-Jolly bodies found in animals from polluted areas in comparison to un-polluted localities.

In Bulgaria a study compared species in different sites of the Rila mountain with different altitudes, where they were collected. They found Howell-Jolly bodies in free-living rodents, as well as in *Chionomys nivalis* and *Myodes glareolus*. The authors described that the presence of Howell-Jolly bodies could be resulting in erythrocyte destruction due to the toxic content of cooper found in samples. The results showed genetic damage in rodents from the lower study area, where it was realistic to assume transboundary pollution as higher lead concentrations were found. Animals trapped in localities with higher altitudes presented with fewer Howell-Jolly bodies per 1 000 erythrocytes (Metcheva *et al.* 2003).

	Males	Females
Category 1	16	10
Category 2	13	11
Category 3	9	8

Table 1. Ratios of males and females in dependence on the number of Howell-Jolly bodies in the peripheral blood of *Chionomys nivalis* (x^2=0.40, p=0.81). For detailed explanation of categories see the text.

	Adults	Non-adults
Category 1	14	12
Category 2	15	9
Category 3	9	8

Table 2. Ratios of adults and non-adults in dependence on the number of Howell-Jolly bodies in the peripheral blood of *Chionomys nivalis* (x^2=0.51, p=0.77). For detailed explanation of categories see the text.

	Males	Females
Category 1	6	4
Category 2	4	5
Category 3	2	7

Table 3. Ratios of males and females in dependence on the number of Howell-Jolly bodies in the peripheral blood of *Myodes glareolus* (x^2=2.77, p=0.24). For detailed explanation of categories see the text.

	Adults	Non-adults
Category 1	4	6
Category 2	5	4
Category 3	3	6

Table 4. Ratios of adults and non-adults in dependence on the number of Howell-Jolly bodies in the peripheral blood of *Myodes glareolus* (x^2=0,95, p=0,61). For detailed explanation of categories see the text.

An insignificant dependence was found between the number of Howell-Jolly bodies and mean values of leucocytes and erythrocytes. Small differences were also found between species. In *Myodes glareolus*, a growing tendency of leucocytes with higher values of Howell-Jolly bodies was found, which may be attributable to higher sensitivity in this species, whereas habitats of *Chionomys nivalis* tend to have better conditions, due to their higher upper limit.

Farkhondeh *et al.* (2014) observed leucocytes in the blood of guinea pigs that were exposed to intraperitoneal lead. Animals exposed to lead had significantly higher leucocyte counts. Changes to all parameters exhibited by lead-exposed animals were statistically significantly higher than those before exposure. The higher total white blood cells (WBC) count in Farkhondeh's results agreed with the results of Kadhim Al-Ali and Abdula (2007), where they found a large increase in total WBC count after acute and chronic exposure to lead. They demonstrated that acute high lead exposure can cause serious effects, including death or long-term damage to organ systems. In comparison, normal values of leucocytes and normal numbers of Howell-Jolly bodies found in a present study of *Chionomys nivalis* and *Myodes glareolus* may indicate that the habitat of free-living rodents in the observed locality is relatively unpolluted and exposure to lead is not high.

In the current study of *Chionomys nivalis* we found that the number of Howell-Jolly bodies was independent of lead concentration in vertebrae of *Chionomys nivalis*. In a study by Chassovnikarova *et al.* (2010) Howell-Jolly bodies were counted in samples from a polluted locality where high lead concentrations were recorded. Significant differences were recorded between Howell-Jolly frequencies in animals captured in un-polluted areas compared to individuals sampled from polluted regions. The results showed differences in sensitivity among the collected three species and significant differences in Howell-Jolly frequency in *A. flavicollis, M. arvalis* and *M. macedonicus*. It is interesting to note the study of Sawicka-Kapusta *et al.* (1987) who investi-

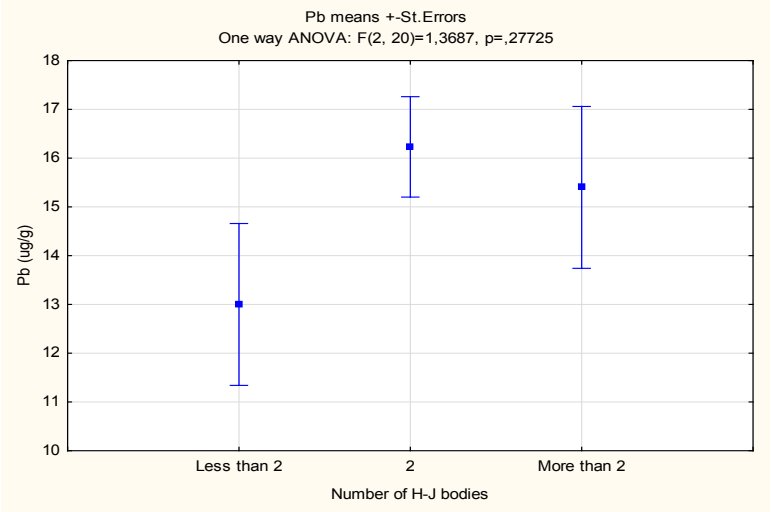

Fig. 1. Amount of lead in bones of *Chionomys nivalis* and the number of Howell-Jolly bodies in their peripheral blood.

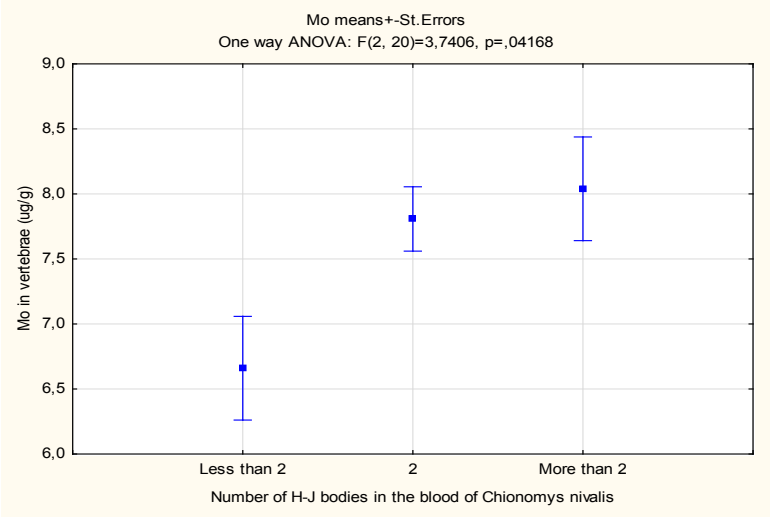

Fig. 2. Amount of molybdenum in bones of *Chionomys nivalis* and the number of Howell-Jolly bodies in their peripheral blood.

gated heavy metal content in rodents living in polluted forests in Poland, recording lead concentrations in *M. glareolus* at significantly higher levels than *A. flavicollis*. In the present study, independence between the number of Howell-Jolly bodies and lead concentration in *Chionomys nivalis* may by the result of low concentrations of lead in the observed locality as well as the relatively high resistance to heavy metals in the alpine zone. Mathieu (1996) came to a similar conclusion in her study, where she described that different species may respond differently to the same exposure level of lead.

In the present study, molybdenum was found to be the most important factor significantly influencing the number of Howell-Jolly bodies in erythrocytes of *Chionomys nivalis*. Molybdenum is a heavy metal, similar to lead, which exhibits similar effects on organisms and impacts on the environment. Molybdenum is an essential element for both plants and animals but high dietary levels can result in molybdenum toxicity in some mammals (Mathieu 1996). Molybdenum compounds are water soluble and are well absorbed through inhalation and oral exposure. The rate of absorption of molybdenum is influenced both by its chemical form and the animal species (WHO 2011). Molybdenum appears the most rapidly in the blood and most organs after gastrointestinal absorption.

The effects of dietary molybdenum (1.7 g/day) were tested in four Holstein cows (Huber *et al.* 1971). After the molybdenum intake was increased to 7 mg/kg of body weight per day, one cow developed severe diarrhoea and exhibited signs of lethargy, cessation of milk synthesis and general emaciation. When the molybdenum dose was increased to 10 mg/kg of body weight per day, two of three cows exhibited these symptoms.

In a study by Miller *et al.* (1956) in which Holtzman rats were fed diets containing hydrogen molybdate at 75 or 300 mg/kg, molybdenum significantly inhibited growth and increased molybdenum concentrations in the liver.

Inhalation studies of molybdenum trioxide were conducted by Chan *et al.* (1998). They found significant exposure-dependent increases in blood molybdenum concentration in exposed rats and mice (Chan *et al.* 1998). Higher concentration of molybdenum in inhaled air was found to significantly increase degeneration of the respiratory system in all exposed males and females compared with controls. The incidence of alveolar/bronchiolar carcinoma was significantly greater in exposed groups of males and females than in the control groups.

Rosoff and Spencer (1964) demonstrated that concentrations of molybdenum in the tissue, bones, and blood rise rapidly after administration of molybdenum compounds. In their study, the effect of water-soluble molybdenum compounds, molybdenum trioxide and calcium molybdate was observed in animals (guinea pigs, rabbits, rats, sheep) after exposure through the intestinal tract.

Asadi *et al.* (2017) investigated effects of molybdenum injected intraperitoneally into Sprague-Dawley rats at different doses of Mo-nanoparticles over a period of 28 days. Hematological and biochemical parameters as well as sexual hormones and histopathological examinations of the liver and testes were assessed and compared with a control group. The results showed that serum levels of testosterone, aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) all decreased significantly with higher concentration of Mo. The histopathological examination of testes showed a decrease in the number of Leydig cells while chronic inflammatory cells increased in portal triad and parenchyma in liver tissue of rats exposed to Mo nanoparticles.

In British Columbia, Mathieu (1996) examined the potential toxic effects of molybdenum in the environment surrounding an active molybdenum mine in small rodents, including the red-backed vole (*Clethrionomys gapperi*), the deer mouse (*Peromyscus maniculatus*), and the meadow vole (*Microtus pennsylvanicus*). Results from this study indicated that molybdenum concentrations in small mammals were not higher in individuals captured in treatment areas than in those which were captured in control areas. She thought that this could be the result of observed toxic effects as the different species of rodents respond differently to the same exposure level of molybdenum and it is also possible that the populations of small mammals found

around the mine area have developed a resistance to the toxic levels of molybdenum over time.

In the present study, increasing numbers of Howell-Jolly bodies were found in erythrocytes as molybdenum concentrations in the tail vertebrae of *Chionomys nivalis* increased. The amount of molybdenum is expected to influence distribution and concentration of molybdenum in the bodies of small mammals and their physiology which may result in changes to homeostasis. Results from the present study showed that higher amounts of molybdenum may change morphology of blood cells and influence the number of Howell-Jolly bodies in peripheral blood, when compared to other studies. This may support the hypothesis that compounds of molybdenum are equally toxic to organisms as other heavy metals, especially for micromammals living in alpine zones, which have a high sensitivity to heavy metal pollution. However, as Mathieu (1996) described, it is possible that these populations of small mammals have developed a resistance to toxic levels of molybdenum and other heavy metals and thus may respond differently to the same exposure level of molybdenum when compared to other species.

Acknowledgements

We would like to thank to Zuzana Hrehová and Michal Némethy for their assistance during fieldwork and their advices, Martina Haas for helped with laboratory analysis and Valika Klementíková for help with laboratory analyses and advised with lots of information. The research was supported by projects ITMS Grant No. 26210120006.

References

Asadi, F., Mohseni, M., Dadashi Noshahr, K., Soleymani, F.H., Jalilvand, A. and Heidari, A. 2017: Effect of molybdenum nanoparticles on blood cells, liver enzymes, and sexual hormones in male rats. *Biological trace element research*, **175**: 50-56.

Balčiauskienė, L., Balčiauskas, L. and Čepukienė, A. 2009: Demographic and morphometric parameters of the yellownecked mouse (*Apodemus flavicollis*) in late autumn-early spring in Lithuania. *Acta Biologica Universitatis Daugavpiliensis*, **9**: 25-34.

Chan, P. C, Herbert, R. A., Roycroft, J. H., Haseman, J. K., Grumbein, S. L., Miller, R. A., and Chou, B. J. 1998: Lung tumor induction by inhalation exposure to molybdenum trioxide in rats and mice. *Toxicological Science,* **45**:58-65.

Çavuşoglu, K., Yalçin, E., Kul, O., Yilmaz, A., Yapar and K. 2010: Detection of genotoxicity induced by heavy metal ions and gamma radiation using micronucleus assay in mice: Pathological evaluation. *SAÜ Fen Bilimleri Enstitüsü Dergisi*, **14**: 1-8.

Chassovnikarova, T., Atanassov, N., Kalaydzhieva, V. and Dimitrov, H. 2010: Micronucleus test from free living rodents as a biomarker for environmental stress in situ. *Biotechnology & Biotechnological Equipment*, **24**: 675-678.

Doubek, J., Bouda, J., Doubek, M., Fürll, M., Knotková, Z., Pejrilová, S., Pravda, D., Scheer, P., Svobodová, Z. and Vodička, R. 2003: Veterinární hematologie. Noviko. Brno.

Evans, H.J., Neary, G.J. and Williamson, F.S. 1959: The relative biological efficiency of single doses of fast neutrons and gamma-rays on *Vicia Faba* roots and the effect of oxygen: Part II. Chromosome damage: The production of micronuclei. *International journal of radiation biology*, **1**: 216-229.

Farkhondeh, T., Boskabady, M.H., Kohi, M.K., Sadeghi-Hashjin, G. and Moin, M. 2014: Lead exposure affects inflammatory mediators, total and differential white blood cells in sensitized guinea pigs during and after sensitization. *Drug and Chemical Toxicol*ogy **37**: 329-35.

Huber, J.T., Price, N.O. and Engel, R.W. 1971: Response of lactating dairy cows to high levels of dietary molybdenum. *Journal of Animal Science*, **32**: 364–367.

Ieradi, L.A., Cristaldi, M., Mascanzoni, D., Cardarelli, E., Grossi, R. and Campanella L. 1996: Genetic damage in urban mice exposed to traffic pollution. *Environmental Pollution,* **92**: 323-328.

Jančová, A., Massányi, P., Naď, P., Skalická, M., Koréneková, B. and Drábeková, J. 2004: Obsah ťažkých kovov v orgánoch drobných zemných cicavcov z emisne zaťaženej oblasti. In: *Rizikové faktory potravového reťazca IV*. pp. 90-94. Slovenská poľnohospodárska univerzita, Nitra.

Janiga M., Hrehová Z., Kostková-Zelinová, V. 2012: Seasonal effects of lead uptake by snow vole *Chionomys nivalis* (Martins, 1842) in West Tatra Mts: Bone metal concentrations and hematological indices. *Polish Journal of Ecology*, **60**: 611-619.

Jarup, L. 2003: Hazards of heavy metal contamination. *British Medical Bulletin*, **68**: 167-182.

Kadhim Al-Ali, S.J. and Abdula, A.F. 2007: Effect of acute and chronic lead nitrate exposure on some physiological parameters in experimental mice. *Basrah Journal of Veterinary Research*, **6**:78-88.

Lovett, G.M. and Kinsman, J.D. 1990: Atmospheric pollutant deposition to high-elevation ecosystems. *Atmospheric Environ*ment, **24**: 2767-2786.

Mathieu, S.F. 1996: Toxic effects of molybdenum on forest small mammals at the Endako mine in north-central British Columbia. Master Thesis. Department of Biology University of Saskatchewan, Saskatoon.

Metcheva, R., Teodorova, S. and Topashka-Ancheva, M. 2003: A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria I: monitoring points and bioaccumulation features. *Ecotoxicology and Environmental Saf*ety, **54**: 176-187.

Metcheva, R., Belcheva, M. and Chassovnikarova, T. 2008: The snow vole (*Chionomys nivalis*) as an appropriate environmental bioindicator in alpine ecosystems. *Science of the Total Environment*, **391**: 278-283.

Miller, R.F., Price, N.O. and Engel, R.W. 1956: Added dietary inorganic sulfate and its effects upon rats fed molybdenum. *Journal of Nutrition*, **60**: 539–547.

Rosoff, B. and Spencer, H. 1964: Fate of molybdenum-99 in man. *Nature*, **202**: 410–411.

Rostal, M.K, Evans, A.L, Solberg, E.J and Arnemo, J.M. 2012: Hematology and serum biochemistry reference ranges of free - ranging moose (*Alces alces*) in Norway. *Journal of Wildlife Diseases*, **48**(3): 548–559.

Sawicka-Kapusta, K., Gorecki, A. and Lange, R. 1987: Heavy metals in rodents from polluted forest in southern Poland. *Ekologia Polska*, **35**(2): 345-354.

Tapisso, J.T., Marques, C.C., Mathias, L. and Ramalhinho, G. 2009: Induction of micronuclei and sister chromatid exchange in bone-marrow cells and abnormalities in sperm of Alegrian mice (*Mus spretus*) exposed to cadmium, lead and zinc. *Mutation Research*, **678**(1): 59- 64.

Topashka-Ancheva, M., Metcheva, R. and Teodorova, S. 2003: A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria II: chromosomal aberrations and blood pathology. *Ecotoxicology and Environmental Safety*, **54**: 188-193.

WHO (World Health Organization) 2011: Guidelines for drinking-water quality. Molybdenum in drinking-water. WHO, Geneva. Online: http://www.who.int/water_sanitation_health/dwq/chemicals/molybdenum.pdf (retrieved 20.4.2017).

Received 23 September 2017; accepted 10 March 2018.