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The morphometric analysis of red blood cells of snow voles *Chionomys nivalis* considering ecotoxicological factors

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Abstract. Blood is an important tissue for studying body processes and also reflects an organism's response to environmental conditions. In this study, we focused on determining whether the size and shape of red blood cells correlates with a selection of ecotoxicological parameters found in snow voles *Chionomys nivalis*. The research was conducted in the High Tatra Mountains (the West Carpathians, Slovakia) in two locations: Dolina Bielych plies (2009, 2010, 2016, 2017) and Brestová (2009, 2010) between April and December, over several years. The concentration of lead found in tailbone samples and mercury found in fur samples does not appear to be significant in the determiniation of size and shape of red blood cells. Likewise, there was no correlation between the 15 elements tested (Sr, S, Cl, Mn, Mo, Ba, Fe, K, Rb, Ca, Zn, Cr, Ti, Ni, Sb) and erythrocyte morphology.

Key words: Chionomys nivalis, erythrocyte size and shape, high altitude, ecotoxicology

Introduction

The alpine environment, with its specific conditions, selectively affects organisms, thereby limiting species diversity. As in other habitats, living organisms are exposed to ecotoxicological effects through water, air or food intake. These conditions represent severe physiological stresses for both animal and plant populations (Rundel and Millar 2016), thus animals inhabiting ecosystems at high altitudes adapt to the harsh conditions of their alpine environment. These physiological changes often feature genetically-based adaptations and evolve under the influence of natural selection (Storz and Moriyama 2008). Snow voles *Chionomys nivalis* (Martins, 1842) are well adapted to their environment. Small mammals are particularily suitable for studying the effects of pollution as bioindicators (Metcheva *et al.* 2003). In the case of snow voles, their relatively short length of life, the small size of their body, limited territory range, and ease of capture make them excellent candidates as bioindicators (Martiniaková *et al.* 2012; Janiga *et al.* 2012).

Hematological characteristics are suitable indicators of adaptations, physiological status, condition, the health state of animals and their survival, and are progressively used in physiological and taxonomic studies (Wołk and Kozłowski 1989; Milner *et al.* 2003; Shah *et al.* 2007). The blood distributes contaminants into tissues making it suitable for determination of past pollutant events and useful for monitoring pollution in high mobility or migratory species (Roscales *et al.* 2010; Maceda-Veiga *et al.* 2015). Increased muscle capillary density, thin-walled vessels, higher hemoglobin-oxygen affinity, and normal or slightly increased hematocrit, are adaptations characteristic of genetically adapted high altitude mammals (Monge and León-Velarde 1991). The spatially fine-grained environmental variation across altitudinal gradients has important meaning for the relative roles of genotypic specialization and phenotypic plasticity in physiological adaptation (Zhang *et al.* 2007).

Human activity introduces pollution to soils through mining, smelting, industry, agriculture and burning of fossil fuels. The disposal of materials containing heavy metals and pollutants (e.g. paint, electronic waste and sewage), also contributes to the burden of environmental contamination. Mountain ranges form a natural barrier for clouds and atmospheric flow and thus are particularly prone to deposition of atmospheric pollutants due to a considerably higher amount of precipitation (White 1949; Lovett and Kinsman 1990). Long-term exposure to a complex of normal and abnormal levels of elements and heavy metals in the environment poses a potential impact to functional and morphological changes in whole organisms, but also has impacts at the cellular level and poses a significant hazard to humans, animals, and the health of ecosystems (Long *et al.* 2002). Essential elements are present in tissues in differing concentrations. The major elements are found in relatively large amounts (e.g. calcium, potassium, chlorine, sulphur, sodium, phosphorus, magnesium, iron); while trace elements occur at lower concentrations (e.g. zinc, manganese, molybdenum, chromium, copper, iodine, cobalt, selenium). Some of these elements are considered contaminants in organisms (e.g. mercury, thallium, cadmium), and their high concentration is manifested by various disorders. Elements are a part of cell structures and they participate in metabolic processes and are essential for the proper functioning of organs. The toxic effect of elements on an organism manifests mainly when an element is in high concentration. This toxicity depends on the means and duration of exposure, the chemical properties and quantity of said element, bioaccumulation, absorption method, as well as the age, gender, genetics and nutritional status of exposed individuals (Fargašová 2008; Tchounwou *et al.* 2012).

In this study compare the morphometric analysis of red blood cells and the potential impact of element concentration (Pb, Sr, S, Cl, Mn, Mo, Ba, Fe, K, Rb, Ca, Zn, Cr, Ti, Ni, Sb) accumulated in bone tissue, as well as mercury accumulated in the fur of snow voles.

Material and Methods

Study area

Samples were collected in the High Tatras Mountains, Dolina Bielych plies (N: 49° 13´ 25.87"; E: 20° 13´ 16.92"; 1673 m a.s.l.). This area is located in the moraine under the southern wall of Jahňací peak. The second sampling site was in the Western Tatras in Brestová (N: 49° 13´ 29.43"; E: 19° 40´ 46.07"; 1902 m a.s.l.). Brestová is located in the main ridge of the Western Tatras, in the western part of the massif Salatin (2047.5 m a.s.l.), from which Brestová is separated by the saddle of Parichvost (1855.5 m a.s.l.). This research includes samples collected April through December, between 2010 and 2017.

Sampling

Animals were captured using Sherman traps. Traps were baited with fresh apple as a water supply, oat flakes, and peanut butter, and then supplemented with straw or sedge to support thermoregulation. At each sampling, approximately 90 traps were set for 2-4 consecutive days. Trapped animals were identified by toe-clipping code (Gurnell and Flowerdew 1990). Blood samples were taken in anesthesia with a solution of isoflurane from the orbital sinus. Blood smears were performed immediately after collection. A piece of the tail approximately 3mm long was also sampled during anesthesia.

Laboratory analysis

In the laboratory, blood smears were stained according to Pappenheim (Doubek *et al.* 2003) and microscope scanned under 1000x magnification. For each investigated animal 100 erythrocytes were randomly chosen (barring deformities) and three measurements were taken using using LAS (Leica Application Suite; ver. 4.5.0; Leica Microsystems CMS GmbH, Switzerland): circumference, longest diameter, shortest diameter.

Statistical analysis

In this study, we correlated the size (circumference) of red blood cells (RBCs) and shape (longer diameter/shorter diameter ratio index) of RBCs with presence of elements (Pb, Hg, Sr, S, Cl, Mn, Mo, Ba, Fe, K, Rb, Ca, Zn, Cr, Ti, Ni, Sb). Results from previous studies for the same animals were used to form our correlations (see Janiga *et al.* 2016; Dúhová 2018; Martinková 2018). Lead content in tail bones was determined by electrothermal atomic absorption spectroscopy (AAS Perkin Elmer 1100B, Norwalk, Connecticut, USA) equipped with deuterium background correction and an HGA 700 graphite furnace with automated sampler AS-70. The procedure and results are published in Janiga *et al.* (2016). Mercury concentrations in fur obtaining from the tail after drying was evaluated by the mercury analyser DMA-80, Milestone, USA. This procedure and results are published in Martinková (2018). To determine the chemical composition in bones of snow voles we sampled part of the tail and analyses were done by X-ray fluorescence, using the hand-held XRF Spectrometer DELTA - Olympus Innov-X. The procedure and results are published in Dúhová (2018). All analyses were perfomed using the Statistica software, Ver. 12., and the F test was used. The significance level was set at 0.05.

Results and Discussion

We found that the size and shape of red blood cells did not correlate with the content of elements and lead found in the tail bone, nor with the content of mercury detected in the fur listed in Table 1.

Organisms require a variable spectrum of element concentrations for life processes, but excessive levels can have a negative impact on the body. In small mammals living in highly polluted areas, chemicals and metals can cumulate in organs and have a negative impact on organisms (Tete *et al.* 2015). Active or passive exposure to metals can hinder developmental stages in organisms (Serbaji *et al.* 2012). Small mammals are suitable for studying the effects of pollution as bioindicators because their distribution is wide, they represent an intermediate stage between low and high trophic levels, their food is varried, they serve as a food source for carnivorous mammals and birds, and take part in different subsystems (Metcheva *et al.* 2003).

Hypoxia, toxicity, and dehydration caused by environmental parameters in polluted areas can produce hematological changes (Gorriz *et al.* 1996). As blood is responsible for inter-tissue redistribution of contaminants, it is suitable for determining past pollutions events and is useful for monitoring pollution in a highly mobile or migratory species (Roscales *et al.* 2010; Maceda-Veiga *et al.* 2015). The ingestion of high quantities of lead, chromium, copper, and cadmium cause anemia, because the blood cells are destroyed (Kanu *et al.* 2006).

The correlation between red blood cell size and shape with element concentration, lead in tail vertebrae, and mercury detected in the fur of snow voles was not significant (Table 1). This is likely because the elements present in bone tissue and mercury retained in fur are stored over a longer period, and are therefore unrelated to current-state blood parameters in this species. There are many studies in which environmental pollution affected organisms where this effect is reflected in their hematological parameters (Gorriz *et al.* 1996; Rogival *et al.* 2006; Tete *et al.* 2015; Waghmare *et al.* 2015). Gorriz *et al.*

Character	Size (circumference)				Shape (L/S diameter)			
	R	p	n	Signif-icancy	R	p	n	Signifi-cancy
Pb (µg g-1 dry weight in bone)	0.2100	0.240	32	NS	0.0300	0.880	32	NS
Hg (mg/kg in fur)	0.1800	0.260	36	NS	0.2700	0.110	36	NS
S (Ppm)	0.3106	0.095	30	NS	-0.0135	0.943	30	NS
Cl (Ppm)	0.0300	0.884	26	NS	0.0106	0.959	26	NS
K (Ppm)	-0.1897	0.315	30	NS	0.1952	0.301	30	NS
Ca (Ppm)	-0.0852	0.654	30	NS	0.2049	0.277	30	NS
Ti (Ppm)	0.2953	0.285	15	NS	0.2783	0.315	15	NS
Cr (Ppm)	0.1847	0.338	29	NS	0.0081	0.967	29	NS
Mn (Ppm)	0.2185	0.246	30	NS	0.1581	0.404	30	NS
Fe (Ppm)	0.1979	0.294	30	NS	-0.0172	0.928	30	NS
Ni (Ppm)	-0.0790	0.689	28	NS	-0.0050	0.980	28	NS
Zn (Ppm)	0.0534	0.779	30	NS	-0.0607	0.750	30	NS
Rb (Ppm)	-0.0866	0.649	30	NS	0.0949	0.618	30	NS
Sr (Ppm)	-0.1213	0.539	28	NS	0.0929	0.638	28	NS
Mo (Ppm)	0.1956	0.300	30	NS	0.0302	0.874	30	NS
Sb (Ppm)	0.4280	0.250	9	NS	0.4384	0.242	9	NS
Ba (Ppm)	-0.1344	0.513	26	NS	-0.1152	0.575	26	NS

Table 1. Morphology of snow vole red blood cells concerning selected ecotoxicology and hematologic characters.

(1996) examined *Apodemus sylvaticus,* L. (wood mouse) and *Mus musculus,* L. (mouse) in a polluted area of Spain. Changes in blood parameters were observed: the value of hematocrit was at a significant decrease and the leucocyte number and mean corpuscular hemoglobin concentration increased. Rogival *et al.* (2006) also observed decreased hematocrit in wood mice found at the most polluted site compared to the reference site. The decrease in values of hematocrit and leucocyte numbers was also observed in wood mice (*Apodemus sylvaticus*) with higher concentrations of Cd in the liver or kidneys (Tete *et al.* 2015). Waghmare *et al.* (2015) studied the concentration of heavy metals present in water and their effects on the hematology of healthy albino rats. In this case the changes in blood parameters were visible. Metal exposure was shown to have a negative impact on the oxygen transport capacity of the blood through a decrease in hematocrit levels (Rogival *et al.* 2006).

In this study, we investigated the ecotoxicological effect of selected elements as a potential burden on changing the size and shape of erythrocytes. However, the results show that the studied elements do not correlate with changes in red blood cell morphometry and these changes are related to other factors.

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