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Multifactorial study on hematology of the yellow-necked mouse (*Apodemus flavicollis*) and platelets as top markers of pollution – field experiments in a chlorinated environment near paper mill industry

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Abstract. Hematological parameters of *Apodemus flavicollis* were analyzed in three field experiments using multivariate statistics. Sample collection was conducted in the vicinity of Ružomberok city, Slovakia, which is the location of a paper mill factory owned by Mondi SCP, Inc. Experiments were designed near the village Lisková, located easterly and situated near the factory, and at the control site on the hill Havran near village Švošov. Animals from the polluted area had higher levels of RBC, Hct, and RBW and lower levels of MPV, and PDW than the control. This ratio between RBC, Hct, RBW versus MPV, PDW did not differ between adult sexes. The independent field experiments clearly confirmed that an increase of RBC, Hct and RDW and a decrease of MPV and PDW is a suitable hematological marker of environmental pollution. We discussed that pollution likely influences the inverse relationship between thrombopoiesis and erythropoiesis. A high erythropoietic state in a polluted area probably induces restraint of thrombopoiesis. The process may be highly influenced by chlorine contamination of the environment. Kidneys and livers contained high amounts of chlorine during the winter period, while in summer the amount of chlorine in kidneys or liver significantly decreased.

Key words: urban pollution, hematology, Apodemus flavicollis, platelets, chlorine

Introduction

There are many pollutants that penetrate into the environment as a result of human activities. The environmental pollution in Ružomberok caused by the Mondi SCP, INC plant was one of the primary reasons for the town being declared an area highly exposed to environmental pollution (Drimal *et al.* 2010). The process of pulp production at Mondi SCP, INC. uses sulphates and other basic chemicals used in production include sodium hydroxide (NaOH) and sodium sulphide (Na_2S). During the processing of organic mass (wood), by-products of sulphur compounds, particularly hydrogen sulphide, dimethyl disulphide, dimethyl sulphide and methyl mercaptan, are released into the environment (Drimal *et al.* 2010). Hydrogen sulphide is a colourless gas, with the distinct odour of rotten eggs (Weil *et al.* 2006; National Research Council 2009). Several studies (Struve *et al.* 2001; Blackstone *et al.* 2005; Gitlin 2008; Al-Magableh *et al.* 2015) assume that exposure to hydrogen sulphide can affect brain neurochemistry, physiology and behaviour in rats (National Research Council 2009). In addition to the sulphates, other pollutants are produced during the papermaking process such as phenols, chlorinated compounds and heavy metals (Gajdoš and Janiga 2015).

Hematological analyses are often used in ecological (Jensen *et al.* 2003; Davis *et al.* 2008) and ecotoxicological studies (Gorriz *et al.* 1996; Bersenyi *et al.* 2003; Tete *et al.* 2015; Maceda-Veiga *et al.* 2015). Blood indices in animals, which live under various conditions and show big differences in their biology, are important to the studies of adaptive mechanisms in mammals (Kostelecka-Myrcha 1967). Hematological characteristics are evolving into increasingly important instruments for the physiological study of mammals, and the physiological response of animals in the damaged area may indicate a physiological stress due to a decreased environmental quality (Pérez-Suárez *et al.* 1990). Hematological parameters can be appropriate indicators of the condition, the physiological status and the immunological resistance of animals (Wolk and Kozlowski 1989). Hematological data is also an important indicator for the condition of individuals and populations of wild animals that are affected by toxins or diseases (Rostal *et al.* 2012). Environmental parameters in polluted areas can produce changes in hematology as a result of contemporary factors such as dehydration, hypoxia and toxicity (Gorriz *et al.* 1996). The erythrocyte count, hematocrit and hemoglobin concentrations indicate the oxygen transport capacity of the blood (Tersago *et al.* 2004), and changes in leukocyte counts can be understood as an index of disease resistance (Rogival *et al.* 2007). According to Kostelecka-Myrcha (1967), respiratory function of hemoglobin is a sensitive indicator of changes in the environment and physiological conditions and can determine adaptive possibilities of the species. Pollutants such as lead or mercury display specific bioaccumulation in blood (Maceda-Veiga *et al.* 2015).

Small mammals are often used as bioindicators of environmental pollution. Due to extensive distribution, wild small mammals have been abundantly used as bioindicators of environmental pollution (Blagojević *et al.* 2012; Talmage and Walton 1991; Shore and Douben 1994; González *et al.* 2008). Small mammals are widely distributed in both contaminated and uncontaminated areas and they accumulate higher amounts of pollutants (Blagojević *et al.* 2012). They are closely adjusted to their environment, and that makes them suitable monitors of pollution and heavy metal concentrations (Sawicka-Kapusta *et al.* 2007). The pattern of toxicant dispersion and levels of heavy metals in different tissues of small mammals are similar to those found in humans (Shore and Rattner 2001). Small mammals are also suitable for biological monitoring because of their small body size, ease of capture, limited range of territory, short life span and their close relation to their environment (Martiniaková *et al.* 2010; 2012).

Hematological analyses of the blood of rodents are widely used as a tool for measurement of environmental pollution. For example, effects of SO_2 and NO_x on hematological parameters were studied in *Apodemus sylvaticus* and *Mus musculus* (Gorriz *et al.* 1996), while effects of ingested Cd, Pb and Hg were studied in rabbits (Bersenyi *et al.* 2003), and albino rats were used to study the effects of heavy metals present in river waters (Waghmare *et al.* 2015). Hematological characteristics of *A. sylvaticus* along a pollution gradient were studied by Rogival *et al.* (2006) and effects of lead and cadmium on hematology of *A. sylvaticus* was studied by Tete *et al.* 2015). Some small mammal populations living in these areas, which are under the risk of pollution, can be exposed to a synergic effect of various chemicals. The chemicals consequently accumulate in different organs and can negatively affect the animal organism (Tete *et al.* 2015). Hematological parameters are essential early clinical diagnosis in human and veterinary medicine but may be effectively used to obtain sufficient information on the health status of wild populations of animals (Waghmare *et al.* 2015).

Mice of genus *Apodemus*, which belongs to the family *Muridae* are suitable pollution bioindicators. The yellow-necked mouse (*Apodemus flavicollis*) is one of the most dominant rodent species in Slovakia and is often used in bioindication studies (Martiniaková *et al.* 2010). Changes in physiology are usually related to blood and blood producing organs, genitalia, digestive tract and respiratory apparatus (Jančová *et al.* 2006). The main aim of this study was to examine the potential effects of the paper mill industry on the blood profile of the field experimental animal – *A. flavicollis*. We designed the field spatio-temporal experiment to reveal which hematological parameters or their combination could serve as a suitable marker of environmental pollution.

Material and Methods

Study area and sampling localities

Sample collection was conducted in the vicinity of Ružomberok city, Slovakia, which is in the proximity of the paper mill factory operated by Mondi SCP, Inc. Experiments were designed near the village Lisková, situated near the factory to the east, and at the control site on the hill Havran near village Švošov. The control locality lies approximately 12 km west of the factory but in a similar natural habitat, near the river Váh. At this location, western winds prevail so the control is less influenced by the pollution from the Mondi complex.

Sampling

Animals were trapped using Sherman live traps. Traps were baited with oatmeal, a piece of apple, peanut butter, and hay or dry grass and leaves. Between 70 and 100 traps were used and set up in the late afternoon and checked early in morning for two consecutive days at each locality. Animals were weighted using a spring scale. We measured the length of body (BL), length of tail (TL), ear (EL) and hind foot (FL). The animals were identified by age and divided into two groups: mature adults and immatures. The breeding status was also identified. Gender was determinated by comparing an anal-genital distances.

Sample analysis - hematology

Following measurements, mice were anesthetized by inhalation of isoflurane dripped on cotton wool for 3 seconds. Blood was collected from the orbital sinus by heparinised capillary tubes for microhematocrits (Brand GMBH + CO KG, Germany) and EDTA coated tubes (Microvette CB 300 K2E, Sarstedt AG & Co., Germany) – Fig.1. After the blood was taken we held a small piece of gauze over the eye for few seconds to stop the bleeding. After sample collection, mice were released at the site of capture.

Collected blood samples (December 2015 – April 2016) from 76 individuals were analyzed by the BC-2800Vet Auto Hematology Analyzer (Shenzhen Mindray Bio-medical Electronics Co., Ltd, China). Samples were analyzed up to 4 hours from collection and at room temperature. The following parameters were determinated: white blood cells (WBC), lymphocytes (Lymph), monocytes (Mon), granulocytes (Gran), Lymph%, Mon%, Gran%, red blood cells (RBC), hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concetration (MCHC), red cell distribution width (RDW), hema-

Fig. 1. Blood collection by capillary tube from orbital sinus of anesthetized yellow necked mouse.

tocrit (Hct), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and 3 histograms: WBC histogram, RBC histogram and PLT histogram. For our analyses we used prediluted mode.

Chlorine and potassium analyses

For the purpose of chlorine and potassium examination, 78 mice were trapped under the permission of the Ministry of Environment of the Slovak Republic in 2015 at the locality Lisková. Dead mice were enclosed in plastic bags and put in the freezer. Animals were measured, and sex was determined after the dissection. Dissection was carried out in sterile laboratory conditions. We removed the liver and kidneys. We placed the liver and kidneys in petri dishes and put in the drier (TCH 100) at a temperature of 53° C for 13 hours. Afterward, we homogenized the dried samples to powder using a mortar. Powdered liver and kidneys were placed separately. For the analysis of chlorine and potassium we used the ED - XRF Spectrometer DELTA MA USA - Waltham device. The device was connected to a fully shielded portable workstation and calibrated. We used the CONOSTAN calibration standard with a calibraton correlation coefficient of 0.999833. Calibration error was 0.9 mg/kg. As sample containers, we used cuvettes with 5μm polycarbonate film on the bottom. We used multiple-beam measurement consisting of three beams for 30 seconds repeated three times. The average value was calculated for each sample from these measurements.

Statistical analyses

Hematological and morphological parameters were analyzed using principal component analysis (PCA) of the correlation matrix of original data (STATISTICA 8 - StatSoft Inc., 2008). One-way and two-way analyses of variance (ANOVA) of principal component scores were used to analyse the differences between experimental groups of animals. ANOVA was also used for a comparison of chlorine and potassium levels in the soft tissues of animals. Values of $p<0.05$ were considered as statistically significant.

Results

Multivariate analysis

Hematological and morphological parameters of *Apodemus flavicollis* were analyzed by using principal component analysis, based on the determination of factor coordinates. Six of the most significant factors are listed in Table 1.

Factor 1 reached approximately 23% of total variance. This factor is endogenous and describes mainly the relationship of mean corpuscular hemoglobin concentration and white blood cells compared to red blood cells. There is a tendency for MCHC and WBC to result lower values with more red blood cells and hematocrit and vice versa. In analysis of variance this factor had a significant relationship only within animals' sexes. Males have

Variable	PC1 1	PC 2	PC3 3	PC 4	PC 5	PC 6
WBC [x10^9/L]	**-0.62**	-0.18	-0.51	-0.40	-0.04	0.17
Lymph [x10^9/L]	-0.48	0.014	**-0.82**	-0.07	-0.05	0.06
Mon [x10^9/L]	**-0.64**	-0.15	-0.40	-0.29	-0.03	0.17
Gran [x10^9/L]	0.13	-0.19	**0.73**	-0.27	0.04	0.07
RBC [x10^{12}/L]	**0.54**	0.17	-0.52	-0.27	**0.42**	-0.29
Hgb [g/L]	-0.42	**-0.46**	0.19	-0.17	-0.04	-0.66
Hct %	**0.67**	0.16	-0.35	-0.29	**0.43**	-0.27
MCV [fL]	0.32	-0.22	0.55	-0.48	0.11	0.13
MCH [pg]	**-0.77**	**-0.43**	0.24	0.02	0.05	-0.28
MCHC [g/L]	**-0.83**	**-0.40**	0.10	0.08	0.04	-0.25
RDW%	-0.06	-0.10	-0.02	**-0.71**	**0.37**	-0.06
PLT [x10^9/L]	0.31	0.36	-0.18	**0.53**	-0.11	-0.38
MPV [fL]	0.49	-0.27	-0.21	-0.52	**-0.57**	-0.13
PDW	0.50	-0.15	-0.06	-0.38	**-0.73**	-0.12
BL [mm]	-0.39	**0.71**	0.08	-0.28	-0.14	0.04
TL [mm]	-0.33	**0.65**	-0.10	-0.04	-0.22	-0.08
FL [mm]	-0.32	**0.62**	0.29	-0.39	0.12	0.08
EL [mm]	-0.25	**0.76**	0.21	-0.14	0.03	-0.12
Weight [g]	-0.25	**0.80**	0.21	-0.12	-0.16	-0.24
Variance	23.27	18.40	14.22	11.63	7.90	5.68

Table 1. Component weights of first six principal components (PC factors) of hematological and morphological parameters of *Apodemus flavicollis*.

lower WBC and MCHC values and higher RBC and Hct than female (Fig. 2).

The second factor is related mainly to morphology and describes the variability in the body size of *A. flavicollis*. This factor reached about 18% of variance.

The third factor, presenting a variance of approximately 14%, is unrelated to external conditions but more related to metabolism. It indicates the variability in amount of white blood cell parameters; with the increased number of lymphocytes the amount of granulocytes decreases and vice versa.

Factor 4 mainly denotes the variance in red blood cell distribution width and platelet count. This factor reached a variance of about 11%. Significance was found in the relationship with age of mice (F=5.7348, p=0.01920). Lower RDW and higher platelet values were observed in mature animals.

Factor 5 refers to the variation in red blood cell indices, mainly RBC count and hematocrit, and platelet indices, primarily platelet distribution width and mean platelet volume. Mean platelet volume and platelet distribution width tend to decrease, whilst RBC count and hematocrit increases. The achieved variance was almost 8%. This factor has a great significance in its relationship with different localities, periods, and the breeding status of animals and seems to be a very effective marker of pollution in urban ecosystems (Fig. 3).

Field experiment - polluted and control experimental area

PC1. At the experimental as well as the control area, the ratio of red blood cells (and Hct) and white blood cells (and MCH, MCHC) did not differ between localities (Fig. 2a).

PC5. Mean platelet volume (MPV) and platelet distribution width (PDW) was relatively high at the control locality, while amount of red blood cells (RBC), hematocrit (Hct) and red blood cell distribution width (RBW) decreased in these animals. Conversely, animals from the polluted area had higher levels of RBC, Hct, and RBW and lower levels of MPV, PDW (Fig. 3a). The ratio of RBC, Hct, and RBW versus MPV, and PDW did not differ between adult sexes.

Field experiment - unfavourable season – winter

PC1. Males had higher levels of RBCs and Hct, and lower levels of WBC, MCH, and MCHC than females. The decrease of RBCs and increase of WBCs, MCH, and MCHC in females was significant in the spring (Fig. 2b)

PC5. In animals trapped during winter, the levels of their RBCs, Hct and RBW were increased while MPV and PDW were decreased. In spring, the amount of RBCs, Hct and RBW decreased while MPV and PDW increased (Fig. 3b).

Field experiment - sexual activity

PC1. Sexually active females had lower levels of RBCs (Hct) and higher levels of WBCs (MCH, MCHC) than inactive females. The active or inactive males did not differ (Fig. 2c).

PC5. Sexually inactive mice had relatively more RBCs and higher Hct or RBW and lower level of MPV and PDW than breeding individuals (Fig. 3c).

Chlorine in the liver and kidney in different seasons

Comparable seasonal trends in chlorine and potassium accumulation suggest that the gastrointestinal and urinal tracts of mice are highly influenced by KCl salts. The lowest levels of chlorine and potassium in the liver occurred in the summer period (Fig. 4), while kidneys contained approximately twice as much chlorine and potassium as livers (Fig. 5).

Discussion

Erythrocyte number (RBC), hematocrit (Hct) versus leukocyte number (WBC), amount of oxygen carrying hemoglobin in RBCs (MCH), average concentration of haemoglobin in RBCs (MCHC)

RBC count represents the number of red blood cells per volume of blood, while hematocrit is a percentage of a given volume of whole blood occupied by packed RBCs. Both characteristics were higher in males than females when compared to average concentrations of haemoglobin in RBCs, amount of oxygen carrying haemoglobin in RBCs, and number of WBCs. Hematocrit and hemoglobin concentrations are considered to record the oxygen transport capacity of the blood (Wolk and Kozlowski 1989; Rogival *et al.* 2006; Tete *et al.* 2015). Although RBC count, haemoglobin concentration and hematocrit are often reported together to be generally higher in females than males in rodents (Weiss and Wardrop 2010), our data confirms that female mice in our area probably suffer more from hyperchromic anemia than males. According to Doubek *et al.* (2003) this phenomenon may be related to respiratory abnormalities, when MCHC increase could improve oxygen transport to cells and tissues. In this case, the number of red blood cells is decreased but the cells contain more hemoglobin. The increased values of oxygen carying hemoglobin and number of RBCs were observed mainly in sexually active females in the spring breeding period. White blood cell counts of these females were also proportionally higher when compared to the number of RBCs. Their increase may also indicate disease or anemia in spring-active females (Doubek *et al.* 2003; Tete *et al.* 2015). Our data suggests that breeding females may be more sensitive to the metal pollution from traffic than males, because traffic is very high at both examined areas. Comparably, Nunes *et al.* (2001) observed increased mean corpuscular hemoglobin concentrations in the algerian mouse from metal polluted areas. Moreover, some hematological parameters may be decreased due to gravidity. According to Doubek *et al.* (2003), decreased values of red blood cells and hematocrit may be observed in the last period of pregnancy and first days of lactation. In both study sites, WBC values were proportionally higher in females than males possibly indicating a response to inflammation or infection. Gorriz *et al.* (1996) demonstrated a significant increase in WBC count for *A. sylvaticus* and *Mus musculus*. According to Gorriz *et al.* (1996), inhalation of high values of SO_2 and NO_2 can produce irritation of the respiratory system and cause a decrease in the phagocyte function resulting in the production of antibodies. We observed high

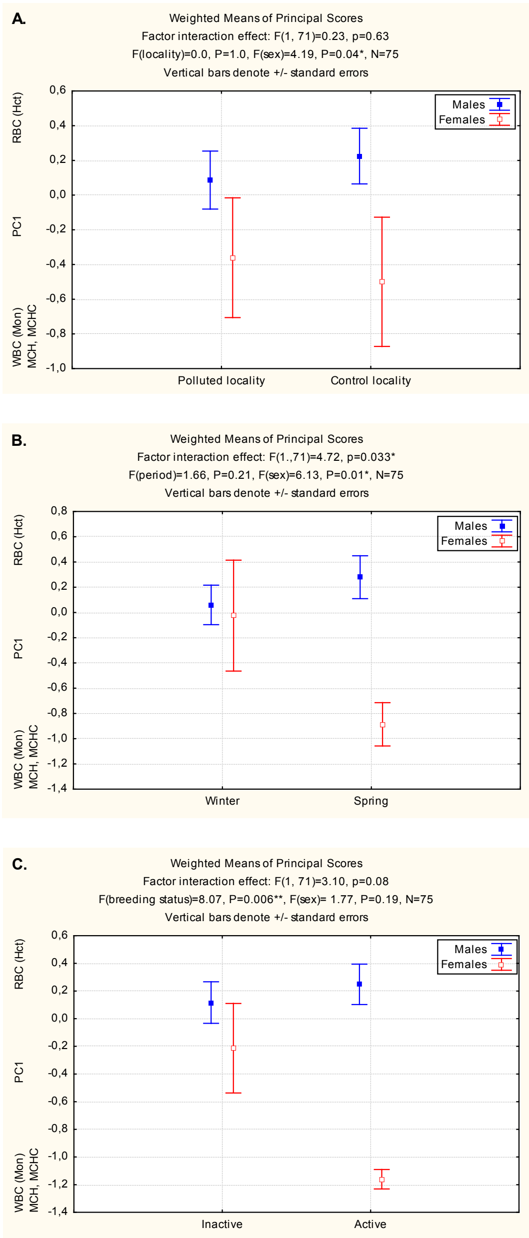

Fig. 2. Significant differences in (WBC (Mon), MCH, MCHC)/(RBC, (Hct)) ratio. Weighted means of principal component scores (PC1) with limits of standard errors. A. Males had significantly higher RBC, Hct and lower WBC, MCH, and MCHC than females. The animals did not differ between localities. B. Ratio of RBCs (Hct) versus WBCs (MCH, MCHC) significantly decreased in females during spring; in males the difference in the ratio was not significant between the seasons. C. Sexually active females contained more WBCs (MCH, MCHC) and less RBCs (Hct) than inactive females. There was a significant difference between the two groups of males.

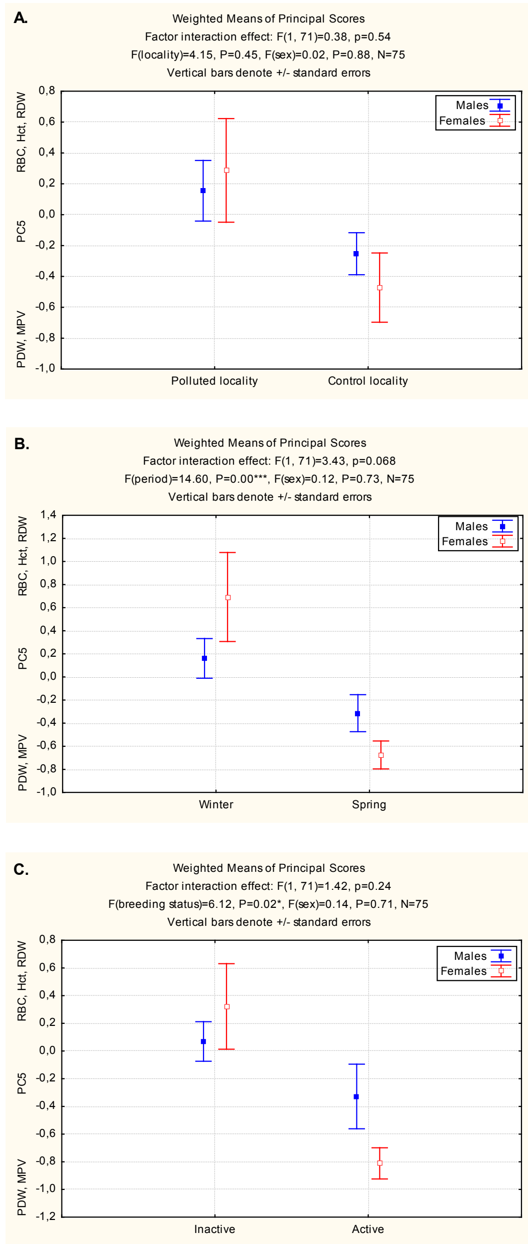

Fig. 3. Significant differences in the (PDW, MPV)/(RBC, Hct, RDW) ratio between mice living at polluted and control locality (A), - between animals trapped in winter and spring period (B), and between sexually inactive and active mice (C). Weighted means of principal component scores (PC5) with limits of standard errors. Animals from polluted area, wintering animals or sexually inactive animals tended to increase the levels of RBC and Hct and decrease PDW and MPV in their blood. The animals did not differ between sexes.

WBC values in both the polluted and the control site, while another study (Tete *et al.* 2015) showed an unexpected white blood cell increment in wood mice for both the polluted and unpolluted site. The authors hypothesized that this increase may be caused by features related to the site, such as unexpected sources of pollution or a high occurrence of parasites. An important factor that may also affect WBC values is stress during the breeding period, which has an influenct on adrenal estrogen. This can increase plasma volume and decrease erythropoiesis (Gorriz *et al.* 1996). Decreased hematocrit values observed in other studies (Bersenyi *et al.* 2003; Tete *et al.* 2015) may indicate an early signalof the potentially adverse effect of metal exposure on the oxygen transport capacity. This may potentially result in heavy metal induced anemia, which could also be caused by the inhibition of globulin synthesis, depression of erythropoiesis or decrease in the level of blood folic acid (Rogival *et al.* 2006; Tete *et al.* 2015; Waghmare *et al.* 2015). Bersenyi *et al.* (2003) indicated that red blood cells, hematocrit and hemoglo-

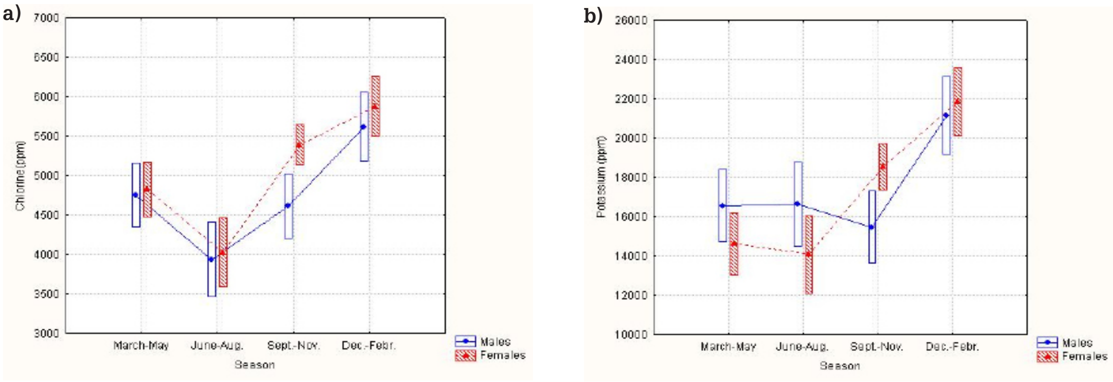

Fig. 4. LS means (+/- standard errors) of concentrations of chlorine (a) and potassium (b) in the liver of *A. flavicollis*. The levels significantly increased from summer to winter, the concentrations did not differ between sexes (Cl - F=1.1, P=0.289, K - F=0.02, P=0.89), and the concentrations equally increased in both sexes from summer to winter (Cl - F(3,68)=0.4, P=0.74, K - F(3,68)=1.2, P=0.33).

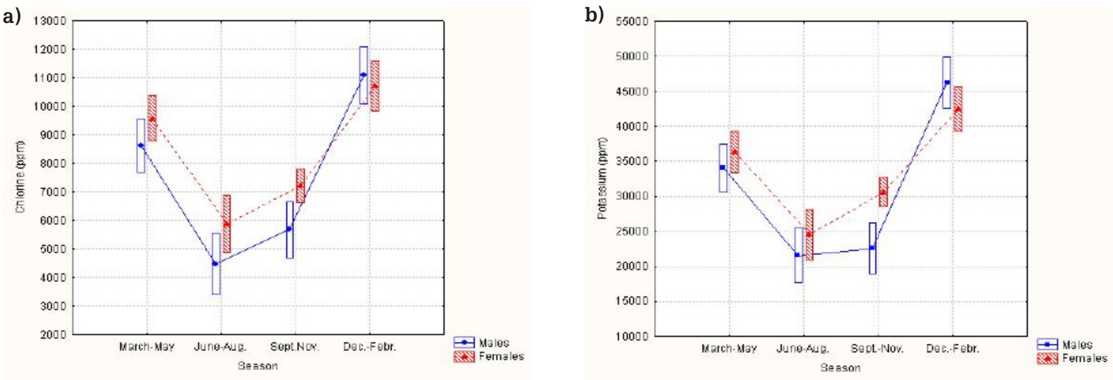

Fig. 5. LS means (+/- standard errors) of concentrations of chlorine (a) and potassium (b) in the kidney of *A. flavicollis*. The levels significantly increased from summer to winter, the concentrations did not differ between sexes (Cl – F=1.84, P=0.18, K – F=1, P=0.32), and the concentrations equally increased in both sexes from summer to winter (Cl – F(3,67)=0.44, P=0.72, K - F(3,67) = 1.14, P=0.33).

bin decreased, while mean corpuscular hemoglobin and mean corpuscular volume increased in rabbits treated with Pb. These results likely demonstrate a higher sensitivity of females to macrocytic anemia than that of males at both our localities. The amount of heavy metal in the soil at both localities is relatively high (Klamárová and Solár 2017), while the main source of metals is supposed to be traffic.

Erythrocyte number (RBC), hematocrit (Hct), erythrocyte distribution width (RDW) versus mean platelet volume (MPV), platelet distribution width (PDW) as an effective marker of pollution

Erythrocyte distribution width (RDW) expresses variation in red blood cells size. RDW is an early indicator of anemia, and also reflects red blood cell fragmentation or dimorphic cell populations. (Wintrobe 2009; Weiss and Wardrop 2010). All three characteristics increased when MPV and PDW decreased. An increase of variable erythrocytes accompanied with smaller (MPV) and less variable platelets (PDW) occurred at the polluted site, in winter and in sexually inactive mice (Fig. 3). The results of our three independent field experiments clearly confirmed that an increase of RBC, Hct and RDW and decrease of MPV and PDW seems to be a very suitable hematological marker of environmental pollution.

The effect of breeding status on hematological parameters was found to be significant. Sexually active animals had higher PDW and MPV values and lower red blood cell counts and hematocrit concentration. According to Doubek *et al.* (2003), some hematological parameters may be decreased due to gravidity. During the last period of pregnancy and first days of lactation, decreased values of hemoglobin, red blood cells, hematocrit, and decreased platelet counts were observed. Decreased platelet counts have an inverse relationship with mean platelet volume (Kostelecka-Myrcha 1967; Doubek *et al.* 2003). As noted by Kostelecka-Myrcha (1967), changes in hematological parameters may also be related to season. The number of blood cells corresponds to temperature, and their diameter changes in relation to the length of days. These variations result in increased hemoglobin function during winter and spring months, when the oxygen requirements are also higher, because of intensive thermoregulation. Sealander (1962) observed the highest values of hemoglobin and hematocrit in deer mice from December to February. Kostelecka-Myrcha (1967) demonstrated that hematocrit has high values in winter months due to the seasonal ability to maintain a high metabolic rate. Acclimation to cold is highly related to caloric intake in animals and is associated with a lack of suitable diet (Sealander 1962).

Pollution and chlorine

We found significant variation in mean platelet volume and platelet distribution width between study sites. Both these indices were higher at the control locality than in the polluted locality when compared to RBCs, Hct, and RDW which lower at the control site. Generally, MPV and PDW values are higher when platelets are activated. Mean platelet volume has an inverse relationship to the number of platelets, and increased count can indicate disease; such as liver disorders. Liver failure is associated with moderate thrombocytopenia (Doubek *et al.* 2003). Decreased MPV is usually recorded in individuals of mammals with the presence of large numbers of megakaryocytes (megakaryocytic hypoplasia) (Wintrobe 2009). Increasing red cell production and decreased mean platelet volume is the primary response to counteract hypoxic stress (Bunn 2013) and it often occurs in the early stages of erythropoiesis. Erythroid lineage takes its origin from a more complex cell lineage differentiation hierarchy, with hematopoietic progenitor cells (Dzierzak and Phillipsen 2013). RBC production is regulated primarily by the peptide hormone erythropoietin (EPO) (Fried 2009). In adult mice, EPO levels regulate RBC output in bone marrow and spleen. Dramatic reductions in RBC numbers lead to compensatory "stress" erythropoiesis (Paulson *et al.* 2011). For example when there is low oxygen pressure or anemia, the spleen of mouse is used to expand the erythropoietic capacity (Socolovsky 2007). EPO is synthesized primarily in the kidneys by peritubular fibroblasts (Jelkmann 2011) and its production in the tissue is directly regulated by tissue oxygen tension through the activity of the hypoxia-inducible transcription factor complex (Bunn 2013). If the hematocrit decreases, the kidney begins to sense low oxygen levels and activate the fibroblasts. Once red cell numbers are restored, EPO production falls to steady-state levels. EPO bears striking homology to thromboietin (TPO), the primary regulator of thrombopoiesis. The two proteins are closely related, sharing 20 % identical amino acids, but there is an inverse relationship between them. A high erythropoietic state induces restraint of thrombopoiesis, and a low erythropoietic state induces an increase in thrombopoiesis (Obama *et al.* 1999). The transcription factor MYB plays a key role in the hematopoietic progenitor cell's lineage choice by enhancing erythropoiesis at the expense of megakaryopoiesis or vice versa (Bianchi *et al.* 2015). Our results suggest that some type of environmental pollution may play an important role in this lineage choice process.

Kidneys and liver are suitable organs to act as indicators of environmental pollution. In winter and early spring, we found unusually high levels of chlorine and potassium in the livers and kidneys of mice trapped near the paper factory. In summer, the levels of chlorine were significantly lower than in winter while the levels of total sulphur in mouse kidney and liver were higher in summer than in winter (Gajdoš and Janiga 2015). As a result we considered chlorine to be a very dangerous pollutant in the region. Several nonlethal effects of chlorine have been demostrated in birds and mammals, including elevated hematocrit values (Turovski 1998). Chlorine dioxide is a potential alternative to using gaseous chlorine for water disinfection. Since chlorine dioxide and ozone are used as a replacement for chlorine gas in the bleaching of wood pulp for paper production in the Ružomberok paper production complex, chlorates and chlorites could also be present in pulp and paper mill wastewater and air. Inorganic chlorates and chlorites can enter foods because they are formed in water as a result of treatment with chlorine dioxide (Moore and Calabrese 1982). Both can produce methemoglobinemia, and chlorite produces hemolytic anemia. Chlorinated phenols are also produced by kraft pulp mills, and some of them are easily absorbed by the gastrointestinal tract of rodents, as indicated by urinary excretion data. Many of them are genotoxic and carcinogenic. High levels of potassium in the liver and kidneys of mice in winter suggest that an unusually high level of KCl salt is likely present in mouse gastrointestinal and urinal systems in this industrial area (cf. Murai *et al.* 2010). However, as discussed above, the primary site for toxicity is the hematopoietic system (Moore and Calabrese 1980). Chlorine dioxide and ozone present major sources of uncertainty because their by-products have not been completely identified. Chlorine dioxide may produce trihalomethanes in the process of chlorination. Trihalomethanes warrant considerable attention, as they appear to be carcinogenic (Turovski 1998).

Several studies were done in the Ružomberok region using a variety of samples, including: rain water and snow (Kučera *et al.* 2016; Švancárová *et al.* 2016); soils (Klamárová and solár 2017); mosses (Vigaš and Šoltés 2013); leaves and needles of trees (Korčeková *et al.* 2015; Grešíková and Janiga 2017); apples (Hudák *et al.* 2015); tit nestlings (Brnušáková and Janiga 2014); and mice (Husáriková *et al.* 2015, Gajdoš and Janiga 2015). These all show that the local ecosystem near the paper mill complex is highly chlorinated mainly in late autumn, winter and early spring periods. For example, the amount of chlorine in the snow ranged from 1 to 30 mg/l, and from 2 to 33 mg/l in rain water in the village Lisková, while the amount of NaCl in the snow ranged from 3 to 48 mg/l and 1 to 38 mg/l in rain water (Švancárová 2016 – databases). Comparatively, the amount of Cl found in streams of the Tatra National Park ranges from 0 to 1.2 mg/l (Gura *et al.* 2013). The amount of total chlorine measured in snow and rain water ranged from 215 to 562 ppm near the factory (Kučera *et al.* 2016 – database). Needles of *Abies alba* contained approximately 600 ppm of chlorine in summer, and 900 ppm in winter. Besides mammals (mice), other high accumulators of chlorine include birds. Hatchlings of great tits contained approximately 16,000 ppm of chlorine in liver tissue and 30,000 ppm in muscle tissue. Fledlings contained approximately 3,000 ppm in muscles and liver. Eggs are laid by females in early spring, but nestlings are fed starting in late April and May when the new fresh diet is available. Young birds are able to secrete elements into growing feathers and bones or to eliminate them via excrement or by storing them in the uropygium and salt gland (Dauwe *et al.* 2002; Deng *et al.* 2007).

The study of hematology in small mammals can serve as a good non-lethal indicator of the environment. The analysis of morphology of red blood cells and their abnormalities that can evoke changes in the health status of organisms in polluted environment will be an important area of study going forward.

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