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Site- and age-related changes in blood count parameters in *Apodemus flavicollis* during short-term monitoring

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Abstract. Blood count values vary between individuals of the same species due to individual predispositions, as well as in response to exogenous factors. In addition to individual components of blood count, red blood cells respond to these environmental changes by their size and shape. However, these changes are less visible and occur more slowly. In this study, we focused on comparing the blood counts of yellow-necked mice (*Apodemus flavicollis*) during a month-long study (August 2018) in the vicinity of the city of Žilina. The first group of individuals came from the polluted site of a heating plant tailings impoundment, this is a waste fly ash storage site. The control site was selected based on habitat similarity approximately 1 km from the tailings pond. Morphological parameters of individuals, blood count parameters and erythrocyte size (cell length, cell width) were analyzed using principal component analysis (PCA). The resulting factor score was compared with location, age and sex. While no significant differences were found by gender, locality and age were significant variables that correlated with the factors. Animals at the tailings site showed higher HGB, MCHC and PLT values (Factor 1) and similarly, animals at the tailings site were smaller, had fewer granulocytes and had higher lymphocyte values (Factor 2). Age was significantly related to Factors 2 and 4. Two-year-old animals were larger, had fewer granulocytes, and more lymphocytes (Factor 2). Two-year-olds with longer paws and longer ears have higher MCH and MCV, but fewer WBCs (Factor 4). Although RBC size and width were related to two factors found (Factors 3 and 6), these factors did not related with location, age or sex..

Key words: yellow-necked mouse, blood count, pollution, age

Introduction

Animals are often used in the investigation and assessment of adaptative mechanisms in the environment, and thus, their blood count values are important in the overall assessment of these changes. Analysis of blood parameters is one of the simplest ways to assess the health and physiological status of a wild vertebrate population (Ardia and Schat 2008; Maceda-Veiga *et al.* 2015; Kophamel *et al.* 2022). Hematological analyses extend ecological (Jensen *et al.* 2003; Davis *et al.* 2008) or ecotoxicological studies (Gorriz *et al.* 1996; Bersenyi *et al.* 2003; Maceda-Veiga *et al.* 2015; Tête *et al.* 2015) and complete a picture of the influence of external factors on the population under study (Ovuru and Ekweozor 2004). External factors include environmental conditions (Večerek *et al.* 2002), elevation (Basak *et al.* 2021), season (Sealander 1964; Ono *et al.* 2021), and pollution (Gorriz *et al.* 1996). Endogenous factors include age, sex and reproductive status, and weight, among others (Doubek *et al.* 2003). Hematological data are also an important indicator of the status of individuals and wildlife populations that are affected by toxins or disease (Rostal *et al.* 2012).

Hematological parameters can be useful indicators of both the physiological state, as well as the condition and state of immunological resistance in animals (Etim *et al.* 2013; Pessini *et al.* 2020). Red blood cell (RBC) parameters reflect the oxygen-carrying capacity of the blood, (i.e., the general metabolic ability of the organism (Wolk and Kozlowski 1989)).

The red blood cell count, concentration of hemoglobin and hematocrit (HCT), and red blood cell distribution width (RDW) reflects the oxygen-carrying capacity of blood (Wolk and Kozlowski 1989; Pérez–Suárez *et al.* 1990; Tersago *et al.* 2004). The RDW is a measure of the extent of variability in RBC volume (Nah *et al.* 2018). However, some disorders cause significantly increased variability in RBC size. Higher RDW values indicate greater size differences (Nah *et al.* 2018). RDW test results are often used in conjunction with mean corpuscular volume (MCV) results to determine the causes of anemia. Variations in red blood cell size (anisocytosis) can be quantified and expressed as red blood cell distribution width (RDW) or as an index of red blood cell morphology. Hemoglobin (HGB) is an iron-containing protein that binds oxygen and is found in red blood cells (erythrocytes). When hemoglobin is deficient, there is inadequate tissue oxygenation (Sakalová *et al.* 1995). The most common cause of anemia is iron deficiency, which leads to reduced heme synthesis. RBCs in iron deficiency anemia are hypochromic (lacking red hemoglobin pigment) and microcytic (smaller than normal). Hematocrit values represent the percentage of RBCs in the blood volume. Higher HCT levels represent a higher capacity for oxygen transport (Birchard 1997), but can also occur in the case of

dehydration, shock, or congenital heart and lung diseases. It may also reflect an increase in red blood cell fraction (e.g., an increase in erythropoietin) or may reflect a decrease in the plasma component of the blood (Dean 2005). Conversely, a low hematocrit represents a decrease in RBC production in the bone marrow, consistent with bone marrow disease (damage from toxins or cancer) or a decrease in erythropoietin, a hormone secreted by the kidneys that stimulates RBC production (Sakalová *et al.* 1995). The mean corpuscular volume (MCV) is the average volume of RBCs. It is part of the blood count and one of the values that indicates anemia. It is occasionally found in acute conditions such as blood loss or hemolysis (Doubek *et al.* 2003). Mean corpuscular hemoglobin (MCH) or "mean cellular hemoglobin" is the average mass of hemoglobin per RBC in a blood sample. It is provided as part of a standard complete blood count. The MCH value is reduced in hypochromic anemias. MCH is reduced when HGB synthesis is reduced or when RBCs are smaller than normal, as in the case of iron deficiency anemia. The mean corpuscular hemoglobin concentration (MCHC) describes the average hemoglobin concentration in each volume of RBCs. MCV, MCH and MCHC are referred to as red blood cell indices, and these values are useful in elucidating the etiology of anemias. Red cell indices can be calculated if the values of hemoglobin, hematocrit (packed cell volume), and red blood cell count are known.

White blood cell (WBC) parameters reflect the level of immunological resistance, with extreme values indicating pathological conditions (Wolk and Kozlowski 1989). The number of leukocytes, lymphocytes, monocytes, and granulocytes reflect the immunological resistance of the organism. If their values are extremely high, they indicate a pathological condition (Wolk and Kozlowski 1989). If their values are low, the organism is exposed to infections. WBCs (leucocytes) are divided into several types, with each type having specific functions and morphologically differing from other types of leukocytes (Greer *et al.* 2013). Leukocytes are cells of the immune system that are involved in protecting the body from infectious diseases and foreign agents. They include three main types: granulocytes, lymphocytes, and monocytes (Doubek *et al.* 2003; Jenkins 2008). Granulocytes are further divided into three groups: neutrophils, eosinophils, and basophils.

Platelets or thrombocytes are a component of blood whose function is to respond to bleeding when blood vessels are injured by clumping and forming blood clots (Laki 1972). Changes in platelet count (PLT), mean platelet volume (MPV), and platelet crit (PTC) may be due to inflammatory disease, iron deficiency, or underdevelopment (Weiss *et al.* 2010).

Changes in erythrocyte size and shape in mammals are most often described because of ontogeny and shunting (Kostelecka-Myrcha 1973; Pis 2008; Grenat *et al.* 2009; Starostová *et al.* 2013) or because of seasonal influences (Kostelecka-Myrcha 1967; Van Voorhies 1996; Ruiz *et al.* 2004). Variability in RBC size and shape is also a response to external conditions and metabolism-related changes during an individual´ s annual cycle, as shown by previous studies of avian RBCs (Janiga *et al.* 2017; Janiga and Haas 2019; Haas and Janiga 2020).

Due to their wide distribution, small mammals are often used as bioindicators of environmental pollution (Talmage and Walton 1991; Shore and Douben 1994; González *et al.* 2008; Blagojević *et al.* 2012). They are widespread in both contaminated and uncontaminated areas and accumulate larger amounts of pollutants (Blagojević *et al.* 2012). They are closely adapted to their environment and are therefore good monitors of pollution and heavy metal concentrations (Sawicka-Kapusta *et al.* 2007). They are also suitable because of their small body size, ease of capture, limited range of territory, short lifespan, and close relationship to their environment (Martiniaková *et al.* 2010, 2012). Populations of small mammals may also be exposed to various chemicals. Chemicals accumulate in organs and can have a negative impact on the organism (Tête *et al.* 2015). The reaction of mice from the polluted area may indicate physiological stress due to diminished environmental quality.

Suitable bioindicators of pollution are mice of the genus Apodemus, which belongs to the family Muridae. The yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834) is often used in bioindication studies (Martiniaková *et al.* 2010). Changes in physiology usually involve the blood and blood-forming organs, genital organs, digestive tract, and respiratory system (Jančová *et al.* 2006). The yellow-necked mouse is a common species with a wide range, mainly in the more mountainous parts of western Europe, except for Scandinavia, southern Spain, western France, and Ireland. It is primarily a woodland species, living in the marginal parts of the forest, but it also occurs in scrubland, fences, orchards and plantations. It lives throughout Slovakia, from the lowlands to the alpine zone (Dungel and Gaisler 2002).

The main objective of this study was to determine the size and shape of erythrocytes in *A. flavicollis* individuals and to determine whether these changes in erythrocytes are directly related to other blood parameters, environmental conditions, and individual characteristics of the individuals. The study is an extension of the research on the impact of the tailing's impoundment (a thermal power plant fly ash dump) on the local biota. The results of the research have already been published separately (see Pogányová *et al.* 2022), this study extends the research to erythrocyte morphometry of the *A. flavicollis* population. We assume that the impact of direct pollution of the tailings pond will be reflected in the blood count values. Changes in HGB content and RDW may also be related to changes in the size and shape of blood cells.

Material and Methods

Field sampling

Mice (n = 28) were captured during August 2018 at the Rosina tailings pond (N 49.180450°; E 18.748783°; Žilina district, Slovakia), where waste fly ash from the burning of lignite in the adjacent heating plant is stored. The control site was selected at approximately 1 km from the tailings pond (N 49.170783°; E 18.747583°) in a forest-meadow

habitat. At the control site, 33 individuals were caught. Capture of animals occurred continuously throughout the month for 4 to 5 consecutive days each week, depending on the weather. Mice were captured using Sherman traps filled with pieces of fruit, oatmeal, and dry grass to provide thermoregulation during the night. The distance between traps was 10 m. Traps were checked in the early morning and evening on each trapping day.

Mice were anesthetized by brief inhalation of chloroform vapor. A cotton swab was dipped in chloroform and placed in a plastic bag, in which the mouse was then placed for a short time. When the activity slowed down and the mouse was put to sleep, it was immediately weighed, and body length, tail length, earlobe length, and hind leg paw length were measured.

Subsequently, blood was collected from the orbital sinus using a hematocrit capillary. Bleeding was stopped with a cotton swab. The age of the mice was determined based on the progression of tooth growth and tooth wear (Wolk and Kozlowski 1989), and the individuals were divided into two age classes, 1-year-old and 2-year-old animals. Morphological measurements also provided information about the age class of the animal. The sex of the animals was determined based on the distance between the anus and the papilla. In males, the papilla is further from the anus, while in females this distance is shorter. In addition, sexual activity was recorded based on the condition of the testes in males and nipples in females.

Laboratory analysis

Collected blood samples (in heparinized tubes) were transported to the laboratory (Institute of High Mountain Biology ŽU, T. Javorina) for further analyses. Samples were analysed 4-5 hours after collection, at room temperature. In the laboratory, a blood smear was made from the blood by smearing on a microscope slide. The remaining blood was used for blood count analysis. All blood samples were analysed using a BC-2800Vet Auto Hematology Analyzer (Mindray Bio-medical Electronics Co., Ltd, China). Blood that was not mixed with anticoagulant was loaded into the analyzer. The instrument determined the folliwing parameters: white blood cells (WBC), lymphocytes (Lymph), monocytes (Mon), granulocytes (Gran), percentage of lymphocytes, monocytes, and granulocytes, red blood cells (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hematocrit (HCT), platelets (PLT), mean platelet volume (MPV), and platelet distribution width (PDW).

Blood smears for microscopic analysis were stained according to Pappenheim (Doubek *et al.* 2003). They were examined microscopically at 1000x magnification. For each individual examined, 50 undeformed erythrocytes were randomly selected, and the following parameters were measured: erythrocyte length (transverse line through the erythrocyte, larger value) erythrocyte width (perpendicular line to the first measurement, smaller value), and erythrocyte perimeter. All measurements were performed using LAS software (Leica Application Suite; ver. 4.5.0; Leica Microsystems CMS GmbH, Switzerland). The slides were viewed meanderingly, and the presence of blood parasites and the general condition of the cells were also monitored. Some cells were affected by hemolysis (cell disintegration).

Statistical analysis

The morphological parameters of the individuals, blood count parameters and erythrocyte size (cell length, cell width) were analyzed by principal component analysis (PCA). Six factors whose percentage of total variance was greater than 5% were selected. For each factor, the dependence on the location (tailing pond, control locality), age (1-year-old, 2-year-old), and sex of individuals was detected. The Kruskal-Wallis H test was used for the analysis, and the confidence level was set at 95% ($p < 0.05$). Statistica software (Ver. 8) was used for the analyses.

Results

Hematology of mice in relation to environment, sex and age of individuals

Principal component analysis (PCA) factor coordinates were calculated based on the individual variables of the study subjects, the mean hematological values of the blood counts and the mean erythrocyte size measured by digital microscopy (Table 1). Five principal factors were selected whose percentage contribution to the total variance was greater than 5%. These factors were further correlated with capture location, sex ,and age of the individual.

Factor 1 (F1) represents a joint increase in the three blood count parameters HGB, MCHC and PLT. Factor 2 (F2) is bipolar and represents a group of animals whose body parameters (body length, tail length and weight) and granulocytes decrease while lymphocyte count increases. Factor 3 (F3) represents the individual erythrocyte shape (length, width), which increases with increasing HCT. Factor 4 (F4) is also a bipolar factor of the morphology of individuals, it is also related to the hematological parameter MCH. When the values of these parameters increase, WBC decreases at the same time. This means that animals with longer paws and ears have higher MCH values, but at the same time have lower WBC values. Factor 5 (F5) is a bipolar factor that is also related to the size of the individuals, this time size is reflected in the size of the paws and tail, and the number of lymphocytes, decreasing if and only if PDW, MCH and MPV increase. Factor 6 (F6) is related to erythrocyte size and shape, is also a bipolar factor, and manifests as an increase in erythrocyte length and width, with a concomitant decrease in monocyte count.

Factors 1 and 2 were significantly related to the location of capture. Animals captured directly at the tailings pond had higher HGB, MCHC and PLT values (F1) than animals from the control site (Fig. 1). Individuals from the tailings site had more lymphocytes, but at the same time fewer granulo-

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Weight	-0.191	**-0.756**	0.070	0.353	-0.176	-0.085
Total length	-0.207	**-0.806**	0.172	0.227	-0.142	-0.056
Tail	0.165	**-0.649**	-0.123	0.423	**-0.337**	-0.107
Paw	0.244	-0.277	-0.048	**0.659**	**-0.402**	-0.066
Ear	0.006	-0.040	0.221	**0.549**	-0.176	-0.059
WBC	0.612	-0.336	0.405	**-0.458**	-0.178	0.049
Lymph #	0.275	0.226	0.636	-0.291	**-0.396**	-0.005
Mon #	0.501	-0.325	0.423	-0.392	-0.195	-0.230
Gran #	0.583	-0.578	0.031	-0.363	0.069	0.093
Lymph %	-0.420	**0.745**	0.243	0.217	-0.293	0.017
Mon %	-0.080	-0.248	-0.100	-0.202	0.005	**-0.565**
Gran %	0.391	**-0.712**	-0.297	-0.173	0.324	0.084
RBC	-0.422	-0.016	0.059	0.055	-0.048	0.185
HGB	**-0.780**	-0.258	0.332	-0.257	0.084	-0.224
HCT	0.228	0.256	**0.529**	0.051	-0.26	0.014
MCV	0.160	-0.041	0.433	0.403	0.248	-0.260
MCH	0.179	-0.180	0.209	**0.493**	**0.510**	0.094
MCHC	**-0.798**	-0.281	0.324	-0.236	0.078	-0.193
RDW	0.518	-0.202	-0.113	-0.028	0.038	0.162
PLT	**-0.722**	-0.274	0.319	-0.140	0.067	-0.271
MPV	0.434	0.347	0.385	0.037	**0.376**	-0.294
PDW	0.461	0.262	0.359	0.323	**0.520**	-0.340
PCT	0.486	0.203	0.431	0.082	-0.232	0.019
Cell length (mean)	-0.215	-0.229	**0.643**	0.019	0.130	**0.513**
Cell width (mean)	-0.321	-0.340	**0.510**	0.078	0.248	**0.494**
Eigenvalue	4.650	4.216	2.992	2.462	1.737	1.519
Total variance	**17.9 %**	**16.2 %**	**11.5 %**	**9.5 %**	**6.7 %**	**5.8 %**

Table 1. Factor coordinates of variables by correlation and percentage of variation in PCA. The most significant factor scores are listed in bold.

cytes and were also smaller (F2, Fig. 2). The second (F2) and fourth factor (F4) were significantly related to the age of individuals. One-year-old individuals were smaller and had fewer granulocytes, but at the same time had more lymphocytes (Fig. 3).

Factor 4 represents a group of individuals that have longer paws and ears and have higher MCH values but fewer WBCs. This factor was significantly related to two-year-old individuals (Fig. 4). No factor was significantly related to the individual's gender.

Discussion

Environmental pollution from anthropogenic sources affects the physiological responses of wildlife, but knowledge of how animals respond to these factors is limited (Gottdenker *et al.* 2014). Levels of hematological parameters are based on the general physiology of the organism, but they also respond to environmental pollution (Gorriz *et al.* 1996). In this study, we focused on the correlation of blood count, morphological characteristics, and erythrocyte size in yellow-necked mouse (*Apodemus flavicollis*), with environmental factors represented by two sites - a tailings pond, which represents a source of pollution, and a control site with no direct source of pollution. We evaluated these variables along with age and sex of individuals.

The variables were evaluated against each other using principal component analysis (PCA) based on the use of a correlation matrix. Using this analysis, it is possible to better compare different relationships between variables, especially when large data sets are difficult to interpret. PCA is a technique to reduce the dimensionality of large data sets, thereby increasing interpretability but minimizing information loss. It does this by creating new uncorrelated variables that gradually maximize the variance. The search for such new variables (principal components) is reduced to solving the eigenvalue/eigenvector problem, and the new variables are defined by the data set, not a priori, making PCA an adaptive data analysis technique. It is also adaptive in another sense, as variants of this technique have been developed to

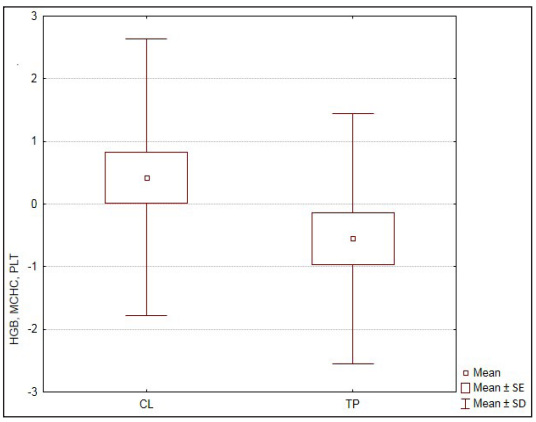

Fig. 1. F1 manifesting based on HGB, MCHC and PLT showed significant differences between sampling sites, with individuals from the tailing pond showing significantly higher values in these blood parameters. KW-H(1;53) = 4.0399; p = 0.0444. CL – control locality; TP – tailing pond; HGB – hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelet.

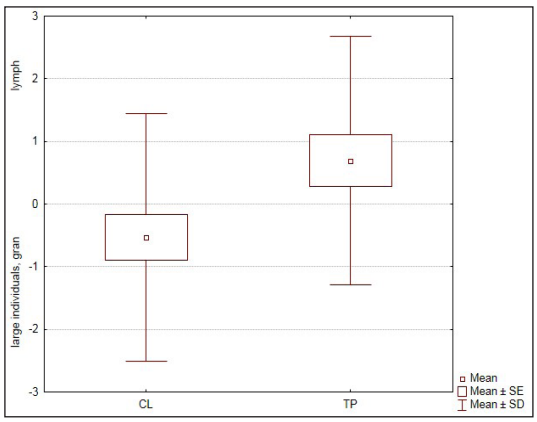

Fig. 2. Animals near the tailing ponds have more lymphocytes but fewer granulocytes and are smaller compared to animals from the control site (F2). KW-H(1;53) = 4.6377; p = 0.0313. CL – control locality; TP – tailing pond, gran – granulocytes; lymph – lymphocytes, large individuals – body length, tail length, weight.

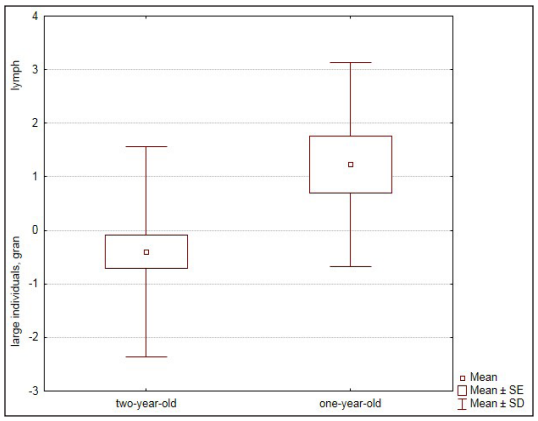

Fig. 3. Difference between ages of individuals on F2. One-year-old animals have more lymphocytes, at the same time they are smaller, and they have fewer granulocytes. KW-H(1;53) = 6.5709; p = 0.0104, gran – granulocytes; lymph – lymphocytes, large individuals – body length, tail length, weight.

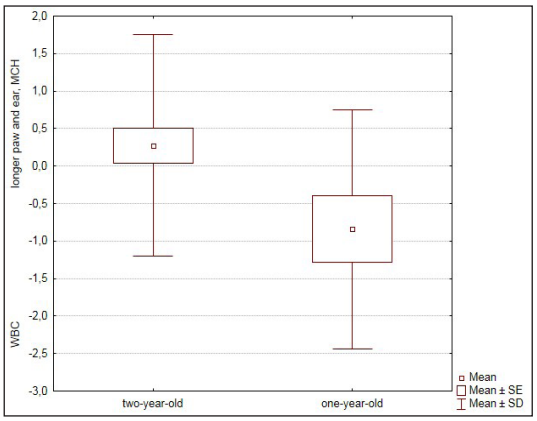

Fig. 4. Difference between ages of individuals and on F4. The older individuals (two-year-old) have greater values of MCH, have longer paws and ears, but have lower values of WBC. KW-H(1;53) = 5.2654; p = 0.0218. MCH – mean corpuscular hemoglobin, WBC – white blood cell.

suit different types of data and structures (Jolliffe and Cadima 2016). In the results of this study, we evaluated the 6 most significant factors for morphometric measurements and blood count results, then compared them to determine the dependence on other variables represented by location, age, and gender of individuals.

Of the six significant factors, only the first two factors (F1 and F2) were related to the sites. We found that animals from the tailings impoundment had higher levels of HGB, MCHC, PLT (F1) than animals captured at the control site. Also, animals at the tailings site had more lymphocytes but fewer granulocytes and were smaller (F2). Some authors have linked the increase of HGB in mice with increased metabolic activity during the winter months (Sealander 1962; Kostelecka-Myrcha 1967). This assumption is closely related to food intake, which requires increased caloric intake (Sealander 1962). Although our research was conducted in summer, when we can rule out an increased energy load due to low ambient temperatures, the increase in HGB may be influenced by an increase in metabolism. A high hemoglobin level is also caused by a low blood oxygen level (hypoxia) that persist for prolonged periods of time. The hemoglobin level is a good measure of the body's ability to carry oxygen. When oxygen is deficient, the body's energy metabolism becomes less economical (Auvinen *et al.* 2021). MCHC is also related to oxygen transfer, and increasing this value could also improve oxygen transfer to cells and tissues. According to Kubota *et al.* (1991), the results confirmed that hemoglobin concentration, red blood cell count, and hematocrit values decrease with increasing age in elderly individuals, and it can be assumed that one of the causes of this phenomenon is decreased protein intake. Since the decline in these blood factors occurs at the control site where there were more two-year-old subjects, these values may also be age related.

Studies (e.g., Rogival *et al.* 2006; Têtе *et al.* 2015; Hondda *et al.* 2017) have shown that when exposed to environmental stress in the form of increasing

concentrations of heavy metals in the environment, tissues and blood in mammals have been shown to cause a decrease in red blood cells, hematocrit, hemoglobin, MCV and/or MCH. Contrary to these claims, our results document an increase in HCT and MCHC in what we considered to be a polluted environment - the tailings impoundment. The tailings pond with stored fly ash from the thermal power plant represents a load in the environment that releases particulate matter (PM) in addition to potential heavy metals into the environment. The study by Siebel de Moraes *et al.* (2020), reported that no significant changes were observed in terms of hematological and biochemical parameters with particulate matter. Based on our results, we can assume that individuals from the control location are metabolically more active, which is related to increased HGB and MCHC values. What other factor this increased metabolism is further related to could be the subject of further research. However, it may also be related to the age of individuals, which conditions other factors discussed below.

The second factor explains that larger individuals have lower levels of lymphocytes and higher levels of granulocytes, thus representing opposite values of agranulocytes and granulocytes. Animals from the tailings pond were smaller, had lower granulocytes but higher levels of lymphocytes compared to animals from the control site. The increase in the number of lymphocytes (T and B lymphocytes) is mainly related to the body's immune response to infections (bacterial, viral, other) and inflammation. An increase in granulocyte count is related to infection, but also to the development of some autoimmune diseases causing persistent (chronic) inflammation. The number of circulating granulocytes also increases in stress situations in birds and mammals (Apanius 1998; Beldomenico *et al.* 2008). In mice, lymphocyte counts can decrease with handling or other stressors (Schwab *et al.* 2005), as well as with age, when neutrophil counts increase (Jain 1993; Boillinger *et al.* 2010), and the number of lymphocytes begins to decrease (Siegel and Walton 2020). Based on these findings, we can assume that the mice from the control site were exposed to more stressful conditions than the individuals from the tailings pond. Since this factor (F2) was also related to age and was significant for two-year-old individuals, we can assume that there were more one-year-old individuals in the group of individuals from the tailings pond. In a study by Tête *et al.* (2015), the authors observed an unexpected increase in white blood cells in wood mice in both polluted and unpolluted sites. The authors hypothesized that this increase may be due to site characteristics such as unexpected sources of pollution or high parasite abundance. Therefore, it is also possible that the increase in lymphocytes is related to factors other than pollution.

Similarly, factor 4 (F4) was also related to the age of the mice. Two-year-old individuals had higher MCH values, longer paws and ears, and fewer WBCs. Older individuals have greater morphometric predispositions than younger individuals, as indicated by F4 (longer paws, ears) and F2 (larger individuals). Although there is an increase in leukocytes with age in mice, this shift is mainly due to an increase in neutrophils; lymphocytes and eosinophils do not show this trend (Boggs *et al.* 1986; Jain 1993). As rodents age, the proportion of lymphocytes decreases, while the proportion of neutrophils increases (Lindstrom *et al.* 2015). Magnani *et al.* (1988) and similarly Restell *et al.* (2014) observed slightly higher lymphocyte counts in older mice than in younger individuals, which contrasts with our results. In addition to diseases related to inflammatory processes, an increase in lymphocytes in the blood occurs with physical exertion or stress (Schwab *et al.* 2005), but also with inadequate intake of substances important for hematopoiesis, such as vitamin B (Jelinek and Koudela 2003). It is therefore likely that although F4 in mice correlates with age, it is also influenced by other factors that were not considered in this research, in particular other environmental conditions or food sources.

The results of our research did not confirm the correlation of the factors with the sex of the individuals. Also, the factors that were correlated with RBCs size (F3 and F6) which we hypothesized to be related to hemoglobin level or hemolysis, were not significantly confirmed. We conclude that changes in cell size and shape cannot be evaluated in a short-term study and given that the lifespan of erythrocytes in mice is approximately 50 days (Khandelwal and Saxena 2007; Makley *et al.* 2010), the factors influencing the change in cell size are manifested over a longer time frame.

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