Oecologia Montana 2019, **28,** 58-69

Moisture regime in *Alnus incana* alluvial forest Javorová valley, Tatra Mountains, the West Carpathians

D. MIHALČIN[1] and J. SOLÁR

Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic; [1]*e-mail: dominikmihalcin7@gmail.com*

Abstract. This study deals with factors adversly influencing the moisture regime of *Alnus incana* at the Javorová valley sampling site. Research was conducted between November 2016 and November 2018. Meteorological variables were evaluated along with measurements taken by a weighable lysimeter and soil probes distributed in the alder stand. Soil moisture was measured using soil probes and a lysimeter at depths of 40 cm, 80 cm and 120 cm. Soil solution samples were taken from the same soil depths as the water well samples from the lysimetric cylinder. From the collected data, five basic factors that influence the moisture regime were determined. We evaluated the changes in the average and monthly values of soil moisture, the monthly and yearly meteorological characteristics, and the seasonal characteristics of the chemical soil solution. The results of the work show that the impact of each individual factor is influenced by the seasonality of the period. At the same time, however, the impact of climate change on individual factors shows their character in the long term. The most important factor influencing the moisture regime is the evapotranspiration of vegetation

Key words: *Alnus incana*, humidity regime, Javorová valley, X-ray, evapotranspiration, lysimeter

Introduction

Water is important for the existence of all living organisms on Earth and exists in all three states - solid, liquid and gaseous. We classify water as a renewable natural resource, but to some extent its circulation is also influenced by humans. The destruction of tropical forests, acid rain, ozone disruption, high usage, melioration and changes in the water regime of soil all disrupt the hydrological cycle. The retention potential of landscapes, ecosystems, or small landscaped areas is of great importance in maintaining biological processes of ecosystems, maintaining biodiversity, and mitigating the effects on human and animal populations (Loreau *et al.* 2001). Significant consequences of climate change are becoming more prevalent. Extreme weather fluctuations have become common; the amount of rain that falls is concentrated in short intervals, and ecosystem stability is at risk due to potential erosion as a result of storm rainfall and flooding, which may also cause extensive property damage (Milly *et al.* 2008). Considering the increasing risk of drought due to global warming, Boczoń *et al.* (2016) examined the direct impacts on the forest ecosystem.

The evolution of forest ecosystems is influenced by multiple factors, whether they are positive or negative phenomena. Climate is the most important factor that significantly affects the development of forest stands. The most significant negative factor affecting the production and growth of tree stands is drought, or water stress. During dry periods, the demand for adaptability increases and may result in native species beginning to grow in new habitats. The consequences of increasing environmental adaptability can be observed today including changes in tree composition or disappearance of some species due to water stress.

Alnus incana (L.) Moench is a species of alder with a wide area of distribution in the cooler areas of the northern hemisphere. It is a relatively short-lived deciduous tree that grows to a height of 15 to 20 m. It is characterized by a shallow root system, a good stump, and root fineness. *Alnus incana* in Slovakia ranges from lowlands to higher mountain locations and has a high demand for sunlight and soil moisture (Bugala and Migas 2011). According to Pagan (1996), it requires habitats with flowing and oxygenated water, and aerated soil such as those a high occurrence of stones. The lifespan of *Alnus incana* has an effect on the developmental cycle of the natural forest. Thanks to the rapid decomposition of fallen leaves with high nitrogen content, alder belongs to the group of meliorating, soil-improving woody plants and also has an irreplaceable function in the biological treatment of watercourses, thanks to its vegetative reproduction, which represents the most economical and appropriate protection (Lukáčik and Bugala 2007).

Kontriš *et al.* (2005) investigated phytocoenoses of marsh-willow shrubs, floodplain forests and slopes of alder in Pieniny National Park, where mountain alder was found predominantly on the alluvium in the mouth of the Dunajec. The relief was irregular and wavy. *Alnus incana* dominated the tree floor, and willows were subdominant. *Sambucus nigra* (L.), *Cornus sanguinea* (L.) and *Lonicera* *xylosteum* (L.) were the most common speciea in the scrub floor. Furthermore, Kontris *et al.* (2005) reported the occurrence of a slope of alder of small size, which were found in the erosion grooves and the slope slides. The tree floor was comprised mostly of *Alnus incana* with minimal *Abies alba* Mill., *Picea abies* (L.) Karst, *Fraxinus excelsior* (L.), *Ulmus laevis* Pallas, *Acer pseudoplatanus* (L.). The scrub floor consisted of species characteristic of field shrub communities, such as *Corylus avellana* (L.), *Prunus spinosa* (L.), *Rubus hirtus* Waldst. & Kit, *Crataegus monogyna* Jacq. The results show that mountain alder along with willows are found on alluvial terraces (Kontris *et al.* 2005). Lukáčik and Bugala (2007), in their analysis of qualitative signs of trunks, crowns and health status of gray alder (*Alnus incana*) and sticky alder (*Alnus glutinosa* (L.) Moench.) in the Laborecká vrchovina state, found that these species are capable of forming natural homogeneous stands. The findings from this analysis pointed to differences within the taxon between individual locations, but also to differences between examined taxons. Growing characteristics natural to the gray alder population (*Alnus incana*) show that production potential of this species is not comparable to other farm trees (Bugala and Parobeková 2016). Producion potential also depends on increasing altitude, where the optimum altitude is 370-400 m a.s.l. (Bugala and Parobeková 2016).

The original alder stands have been significantly influenced by human activities. They have been converted into agricultural land or removed as a result of watercourse modifications. The biological balance and the aesthetic value of watercourses were disturbed by the elimination of alder stands. Shore erosion, loss of wood production, devastation of shoreline vegetation, and other damage to crops were triggered by removal of these trees. According to Lukáčik and Bugala (2009), the proper management of alder forests could contribute to the protection of natural environments and increase the biological value of the landscape.

Stradiot *et al.* (2014) evaluated the spatial variability of retention properties of selected soils in the Borská lowlands. Stradiot *et al.* (2014) state that the granular composition of the soil, the mineralogy of the clay fraction, the properties of organic matter, soil structures and so on, affect the relationship of soil water and soil moisture. The variability of the soil structure affects the water content in the soil and its ability to retain this moisture (Rehák *et al.* 2006). According to Rehák (2006), the size and shape of pores are particularly important for soil and water dynamics. The organic content of soil, soil species, structure and genetic soil horizon all determine pore distribution in the soil profile. Based on the above stated characteristics and the proportion of clay, dust, and sand in the soil, soils are classified into soil species. The basic soil species include gravel soils, stony soils, boulder soils, light soils (sandy, loamy-sandy), medium-heavy soils (sandy-loamy, loamy, loamy-loamy) and heavy soils (clay soils). Stradiot *et al.* (2014) state that "the group of medium-heavy soils shows a relatively smooth course of the drainage branch of retention curve, while in the group of the light soils there is a rapid decrease in moisture".

We describe water balance in forest ecosystems using the quatitative state of the water regime during a period of time in the forest stand. The result is a correlation between the incoming and outgoing water in the environment, which determines the water balance. Changes in hydrological conditions of an environment are manifested as an imbalance between the gain and loss of water in the soil - plant - atmosphere system (Minďáš *et al.* 2010; Střelcová *et al.* 2011). Water balance is determined by the flow of water in and out of the soil. Using soil water balance we can set determine values including transpiration, evapotranspiration, and soil vapor. In cases of excellent structure and abundant overgrowth of roots, forest soils can accumulate up to 200 liters of water per square meter per month, which flows slowly and evenly.

Factors including climatic conditions, altitude, terrain slope, exposure, stand structure, woody composition, age, stifling and canopy affect the water balance (Tužinský 2007; Vida *et al.* 2012). Atmospheric precipitation in all forms, including dew, is the main source of water for forest ecosystems. Depending on volume, frequency and timing during the growing season, rainfall events will affect water balance differently. Horizontal precipitation, particularly fog, has an exceptional significance in terms of water gain in forest ecosystems at higher altitudes. Significant precipitation differentiation occurs when rainfall flows into the forest ecosystem, and when this precipitation comes into contact with vegetation. Interception occurs when rainfall is trapped in trees, shrubs, and herbaceous vegetation. Some rainfall runs down the trunks of trees, and some penetrates the soil's surface (Penka 1985; Tužinský 2007).

Variability in the distribution of rainfall in forest ecosystems is most affected by the tree crowns. Precipitation trapped in varying amounts on the surface of the arboreal, shrubbery, and herbaceous vegetation subsequently evaporates into the air. This process is called interception. From a hydrological point of view, interception is considered part of water loss (when considering total vapor), and is a non-reproductive component of evapotranspiration (Tužinský 2007).

Forest stands can hold between 10-50% of atmospheric precipitation, depending on their composition and stand structure, the developmental stage of the vegetation, the growing season, weather conditions and other factors. The correlation between crown density and interception means that beech and spruce stands (30-50%) retain more rainfall than pine trees (15-30 %) (Krečmer 1962; Tužinský 2007). Drain represents an important income component of water balance for these stands, and smooth-bark vegetation tend to exhibit higher water content along their trunk. Kantor (1983) mentions for that in beech stands, water collected along the trunk can account for 19.9% of free area rainfall, while this value for spruce trees is only 1.4%.

Water transpiration in plants takes place through vents. Transpiration is a physico-biological process and is an important component of water balance expenditure (Penka 1985; Novák 1995). Evapotranspiration is an ongoing process in the environment. Evapotranspiration is the evaporation of water that is consumed by plant transpiration and is increased by the amount of water that evaporates through interception and from soil under tree stands. The forest eliminates wind speed, and shade undergrowth and increases the relative humidity of the air, causing vapor reduction. Forest stands reduce the annual evaporation by half compared to the free surfaces. In the summer months evaporation in the forest is reduced by 70-90% compared to unforested areas.

The aim of this work was to determine factors affecting the moisture regime in the alluvial forest of *Alnus incana* at the selected site of Javorová valley. Partial objectives were:

- evaluation of moisture conditions during the experiment
- evaluation of meteorological characteristics during the experiment
- evaluation of chemical elements of soil solution

Material and Methods

Study area

The study area was located very close to the Institute of High Mountain Biology, which is situated in the village of Tatranská Javorina (N: 49° 16´ 5", E: 20° 8´ 29"). Tatranská Javorina falls into a buffer zone of the Tatra National Park. From a geological point of view, the study area is located on fluvial sediments of mountain streams. Thus, we find a diversity of surface geography, as fluvial sediments in the mouth of the Javorova valley are carbonate (Belianske Tatras) and granite (High Tatras) from the Tatra mountains (Vološčuk *et al.* 1994). Lithium, ranker, podzol, cambis, rendzina, and fluvi type soils have been formed. The whole territory encompassing the Tatras is characterised by typical features of the alpine climate. It is divided into three circuits (subdivisions) according to the average July temperature, between 10° - 12° C. The relative air humidity of mountain areas varies, but temperature inversions (increases in air temperature with altitude) are characteristic of mountainous areas (Smolen and Ostrozlik 1994).

Equipment

We used two weather stations to measure and obtain meteorological data. The first meteorological station - Vantage Pro2 (Davis Instruments, USA) - was located in an open area. The second meteorological station with a weight lysimeter from Umwelt-Geräte-Technik GmbH (Germany) was located at the edge of an alder stand. Both meteorological stations and the lysimeter transmit data using the internet to a central server at the Institute of High Mountain Biology. Data was measured continuously and recorded in 15 minute intervals (mean or sum value of 15 minute intervals). Additionaly, we used soil probes from UGT-Umwelt-Geräte-Technik GmbH (Germany) for monitoring soil temperature and moisture within the alder stand. Leaf moisture sensors from Davis Instruments (USA) were used to monitor the level of surface moisture on foliage. Using the lysimeter, we measured soil temperature (° C), moisture (%) and tension (hPa) at depths of 40 cm, 80 cm and 120 cm, as well as run off (mm), drain off (mm) and weight (kg) of the lysimeter. In the alder stand, we measured soil temperature (° C) and moisture (%) using soil probes, which were placed at the same depths (40 cm, 80 cm and 120 cm) under the root systems in two locations in the middle of the stand. The meteorological stations measured air temperature (° C), humidity (%), pressure (hPa), solar radiation (watt), precipitation (mm), wind speed ($m.s^{-1}$) and wind direction.

Evapotranspiration modeling

Based on measured meteorological variables (air temperature, humidity, pressure, wind speed and radiation) we calculated potential evapotranspiration (PET). We used the FAO Penman-Monteith ET_0 (Allen *et al.* 1998).

Actual evaporation (AET) is determined by the difference in the daily changes of the lysimetric cylinder weights where precipitation and water seepage enter the formula:

$$AET = (W_{i-1} + p_{i-1} - s_{i-1}) - (W_i + p_i - s_i)$$

W	weight of lysymeter	[kg]
P	precipitation	[mm^{-1} $hour^{-1}$]
S	seepage water	[mm^{-1} $hour^{-1}$]
i	date of day	

Water sample collection and determination of chemical elements

Water samples were gathered using tensiometer probes (at 40 cm, 80 cm, 120 cm) to collect run off as well as drain off, and a well sample that should represent the groundwater source at that location. The samples were collected every fourteen days, or twich per month.

Chemical elements were determined and measured by an ED-XRF Spectrometer DELTA (Bas, Rudice, CZECH). Measurement of water samples was carried out in the closed protective box of the ED-XRF Spectrometer DELTA. Water samples were analysed in a special plastic vial and every sample was measured using the same duration of X-ray beam (80 sec.). In the process of sample X-raying, we used the multiple-beam measurement mode calibrated by "Reference Material for Elements in Surface Water - SPS-SW2 Batch 127" (Spectrapure Standards, Norway).

Statistical analysis

A matrix was created in Microsoft Excel for all the data files, with 15 minute intervals. The data matrix was processed in STATISTICA 8.0 (StatSoft Inc., 2008). Data from the lysimeter, soil probe and meteorological stations were analysed using principal component analysis (PCA), with determination of cross-correlation, based on factor scores. For analysis of differences between groups of parameters, ANOVA one-way analysis of variance was used. Values of $p \leq 0.05$ were considered to be statistically significant. Daily inputs of meteorological values were evaluated by ANOVA (one-way analysis of variance) on the base of data with 15 minute intervals.

Results

In tables 1 and 2 we summarize the monthly and yearly rainfall as well as the average air temperatures, soil temperature in at 5 cm, relative air humidity, wind speed, air pressure and solar radiation.

The coldest month of 2017 was January and the hottest month was August. In 2017, the highest average daily air temperature was recorded as 21.37˚ C on 1.8.2017. The lowest average daily air temperature was recorded as -22.28˚ C on 7.1.2017. When compared to the long-term average, the yearly air temperature increased by 1.32˚ C. September was characterized by the largest amount of rainfall (246 mm) and January was characterized by the smallest amount of rainfall. The biggest rainfall events in 2017 occurred on 23.7.2017 (60.00 mm) and 21.9.2017 (60.20 mm), respectively. Overall yearly rainfall was consistent with the long-term average, but in September we recorded an abnormal occurrence of rainfall, when rainfall exceeded the long-term average.

The coldest month of 2018 was February and the warmest month of the year was August (Table 2). The highest average air temperature was 19.25˚ C and was recorded on 9.8.2018, while the lowest average air temperature recorded was -20.04˚ C on 28.2.2018. Measurements in 2018 were completed on 28.11.2018, and excluded the month of December, so we were unable to compare these values with the long-term average for the year. June (244 mm) and July (349 mm) were characterized by the highest amount of rainfall. January, along with Feb-

Month	Rain open space	Rain in forest	Air temperature	Soil temperature in 5 cm	Humidity	Wind speed	Pressure	Radiation
1	5.00	24.10	-8.74	-0.11	79.62	0.97	1020.29	37.07
2	24.20	25.20	-0.78	0.18	79.38	0.89	1017.67	56.36
3	43.20	39.60	2.59	2.79	76.08	1.10	1016.55	77.97
4	143.40	119.80	3.37	3.80	81.51	1.07	1016.14	86.18
5	145.20	90.00	10.04	10.03	82.49	0.97	1018.72	109.87
6	135.60	62.10	14.32	12.76	73.85	1.06	1018.00	100.60
7	151.40	64.20	14.08	12.96	82.40	0.74	1018.29	71.54
8	119.00	99.20	15.40	14.09	79.75	0.64	1022.11	88.28
9	246.00	210.20	9.53	9.65	87.56	0.53	1017.26	53.59
10	117.60	109.60	5.69	6.16	82.46	0.72	1019.37	39.93
11	65.40	107.70	0.19	1.63	89.23	0.40	1015.10	28.73
12	32.60	121.80	-2.09	0.22	84.38	0.75	1014.08	12.09
Year	1228.60	1073.5	5.32	6.22	81.55	0.82	1017.79	63.59

Table 1. . Monthly and yearly meteorological characteristics of 2017 (rain – mm; temperature - ˚ C; humidity - %; wind speed – m/s; pressure – hPa; radiation – W/m).

Month	Rain open space	Rain in forest	Air temperature	Soil temperature in 5 cm	Humidity	Wind speed	Pressure	Radiation
1	4.80	17.10	-2.16	-0.93	85.18	0.59	1015.73	28.42
2	4.40	43.70	-5.40	-0.22	89.68	0.30	1013.77	21.18
3	25.80	25.50	-2.88	-0.62	79.65	0.57	1007.41	78.07
4	47.60	41.50	10.06	8.94	65.56	1.08	1017.38	135.19
5	96.40	62.40	11.84	11.04	78.14	0.94	1018.92	87.08
6	244.00	179.80	12.91	12.20	88.67	0.48	1017.57	44.30
7	349.00	307.50	14.60	13.37	84.90	0.51	1017.39	49.91
8	162.60	119.80	15.61	14.50	83.49	0.51	1021.50	70.00
9	97.20	71.50	11.29	10.81	84.25	0.50	1022.94	56.23
10	80.80	65.10	7.66	7.45	80.93	0.70	1020.54	46.28
11	16.80	24.10	3.17	4.58	83.30	0.37	1021.27	39.53
Year	1129.40	958.00	7.30	7.56	81.87	0.60	1017.71	61.10

Table 2. Monthly and yearly meteorological characteristics of 2018 (rain – mm; temperature - ˚ C; humidity - %; wind speed – m/s; pressure – hPa; radiation – W/m)).

ruary, were characterized by the smallest amount of rainfall. The biggest rainfall events in 2018 were recorded in July; 18.7.2018 (88.40 mm) and 22.7.2018 (69.80 mm). Compared to the long-term average, the months of June and July had an excess of precipitation, where several times more precipitation than the long-term average fell. The evaluation of the yearly total rainfall was not evaluated due to the missing data from December 2018.

Evaluation of soil moisture regime of monitored locations

Changes in values of average monthly soil moisture (Lys H) are consistent in the long term (Fig. 1a). The most significant oscillations are at 40 cm and 80 cm deep. The lowest soil moisture at 40 cm occurred during August. The month of August is also significant in terms of the average monthly temperature since it was the hottest month in both cases during 2017 and 2018.

More significant changes in the average monthly values of soil moisture during the year can be seen in the alder forest in locality 1 (Loc1 H, Fig. 1b). The most significant decrease in moisture occurs at the beginning of the calendar year between January and February, with the subsequent March increase in moisture at the upper soil horizons. Between March and May, the soil moisture decreases very slightly again. From May to July, atmospheric precipitation in the form of rain, which in turn affects and increases soil moisture, occurs increasingly in the stand, particularly in the upper soil horizons. From July to the end of the calendar year, soil moisture is higher than in the spring months, and is relatively constant. At the end of the year moisture starts to increase, which is probably caused by winter snowfall in the form of snow. Lower soil horizons were also affected by rainfall activity in May and June. The highest moisture value at 120 cm was measured in June. Later in the year, the moisture in the lower soil horizons decreased, balancing out by the end of the calendar year. Figure 1b also shows that the month of February and May yielded the lowest levels of groundwater. Medium soil horizons at 80 cm oscillate slightly throughout the year and show no major deviations. A slight decrease in soil moisture occurred simultaneously with a decrease in moisture in the upper soil horizons.

Moisture characteristics at locality 2 in the alder forest (Loc2 H) differed from those in locality 1 (Fig. 2a). Moisture decline in the upper horizon early in the calendar year extended into March, whereas moisture decline only extended into February at locality 1. From May to the end of the calendar year, soil moisture recorded at locality 2 is similar to that of locality 1, but with more significant oscillations and a more significant decline in soil moisture in August. Measurements from the middle soil horizons are missing, which makes it impossible to evaluate them. The most significant changes compared to locality 1 occur at the lower soil horizons (Fig. 2b). The soil moisture profile from both localities is most significant for its oscillations. A decline in moisture occurs in February, May and August. The greatest differences in soil moisture occur between February and March, and the highest moisture values during the year occur in June, July and

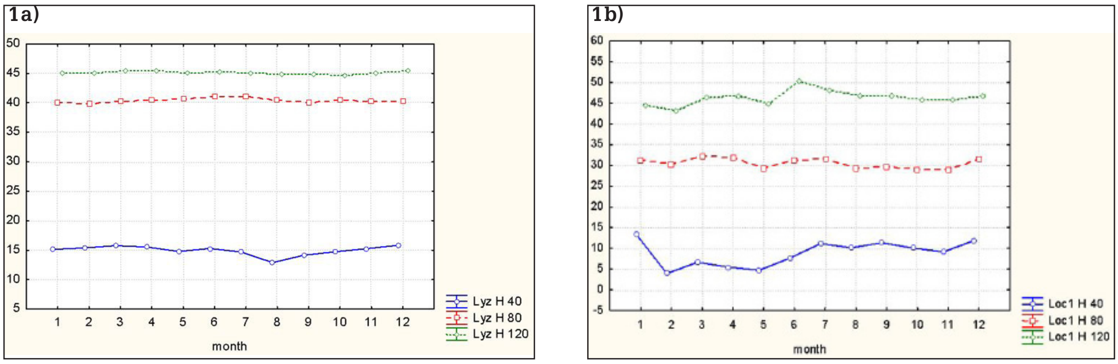

Fig. 1a) Changes in average monthly values of soil moisture in lysimeter during the reference period. **b)** Changes in average monthly values of soil moisture in stand of alder in locality 1 during the reference period.

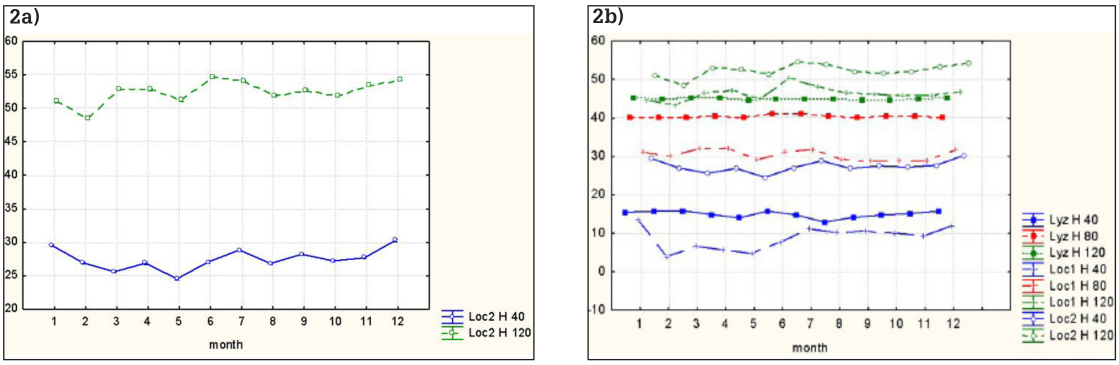

Fig. 2a) Changes in average monthly values of soil moisture in stand of alder in locality 2 during the reference period. **b)** Comparison of changes in average monthly values of soil moisture in the monitored locations during the reference period.

December. The characteristics of each locality is different, so it follows that soil moisture measurements over the course of the year should differ as well. Locality 2 is characterized by the highest soil moisture in both upper soil horizons and lower soil horizons. Locality 1 has the lowest soil moisture in the upper horizons. At the lower soil horizons, locality 1 is slightly drier at the beginning of the calendar year, but soil moisture is shown to increase in March. Moisture levels in the middle layers of soil horizons (80 cm) were lowest at locality 1.

Principle component analysis

The collected data was evaluated using principle component analysis based on determination of factor coordinates, in order to understand the relationships between individual measured characteristics. The most significant were the first five factors (Table 3). The results of the analysis show individual relationships and their seasonal patterns during the year. The first factor with a variance of 34.145% is the most significant. This phenomenon

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
Lyz T 40	**0.981**	0.077	-0.074	0.045	-0.021
Lyz T 80	**0.981**	0.083	-0.039	0.129	-0.005
Lyz T 120	**0.974**	0.087	-0.019	0.170	0.010
Lyz H 40	**-0.526**	**0.529**	**-0.301**	0.047	**-0.287**
Lyz H 80	0.197	**0.620**	**-0.464**	0.075	**-0.321**
Lyz H 120	-0.344	**0.688**	**-0.520**	0.034	-0.048
Lyz tens 120	0.243	**-0.717**	**0.510**	-0.097	**0.205**
Lyz drain sum	0.038	**0.761**	-0.081	-0.130	**0.355**
Lyz Weight	**-0.767**	0.259	-0.118	0.028	-0.182
T 2m	**0.889**	-0.004	-0.242	-0.160	-0.023
H 2m	-0.056	**0.563**	**0.472**	**0.496**	-0.190
Pressure 2m	0.480	-0.287	0.036	0.223	0.186
Radiation 2m	**0.563**	-0.337	**-0.504**	**-0.367**	0.022
rain forest sum	0.133	**0.732**	0.261	-0.297	**0.310**
T soil 5cm	**0.935**	0.000	-0.171	-0.111	-0.021
Lyz H % listy	0.174	**0.703**	**0.400**	-0.136	-0.111
Loc1 T 120	**0.900**	0.100	0.047	**0.320**	0.051
Loc1 T 80	**0.965**	0.092	-0.003	0.200	0.020
Loc1 T 40	**0.984**	0.072	-0.065	0.060	-0.011
Loc1 H 120	0.031	**0.710**	**-0.450**	0.102	-0.185
Loc1 H 80	-0.215	**0.770**	**-0.301**	-0.084	**0.235**
Loc1 H 40	0.000	**0.540**	-0.108	0.163	**0.573**
Loc2 T 120	**0.896**	0.096	0.058	**0.324**	0.035
Loc2 T 40	**0.982**	0.074	-0.045	0.099	-0.009
Loc2 H 120	-0.102	**0.611**	**-0.467**	0.158	-0.102
Loc2 H 40	-0.168	**0.787**	-0.137	0.069	**0.390**
Loc2 H % listy	0.089	**0.651**	**0.479**	-0.057	-0.143
rain building sum	0.247	**0.758**	**0.348**	**-0.378**	0.116
wind Speed	0.057	-0.115	**-0.418**	**-0.548**	0.050
I forest mean	0.288	0.216	0.254	-0.233	**-0.381**
Rain/Drain	0.328	0.388	**0.594**	**-0.434**	-0.170
Weght change	-0.031	**0.480**	**0.492**	-0.058	0.100
AET	**-0.533**	-0.312	-0.197	**0.343**	0.222
ETo	-0.495	-0.397	0.023	-0.025	0.223
ETsum	**0.611**	-0.342	**-0.523**	**-0.395**	0.046
Total variance %	34.145	23.119	10.595	5.602	4.222
Cumulative variance %	34.145	57.264	67.859	73.461	77.683

Table 3. Principle component analysis with first five factor coordinates.

presents the contrast in evapotranspiration during summer and winter. The increase in air temperature, soil temperatures and radiation allow for the spring onset of vegetation and water consumption for plant development and photosynthesis. The most significant manifestation of this factor occurs between May and September. While vegetation is small, evaporation from the soil prevails, but as vegetation grows over time, the volume of water lot is offset by evaporation and plant transpiration. If the vegetation is well developed and the stand is well connected with the treetops, transpiration represents the majority of water lost by the stand (Fig. 3) and the importance of vapor from the soil decreases. The opposite occurs from November to April when the loss of water from stands is the most significant through evaporation from the soil. Transpiration is affected by radiation and temperature. The first factor indicates that the function of evapotranspiration of the forest in Javorová valley is the most significant between May and October.

The second factor, with a variance of 23.119% is an important phenomenon in terms of water balance of the stand. The increase in soil moisture, air humidity and bottom runoff causes an increase in atmospheric precipitation. The increase in atmospheric precipitation is reflected in the change in lysimetric cylinder weight. The most significant second-factor relationships include growing rainfall, growing soil moisture at the 80 cm and 120 cm depths, and decreasing tension at the 120 cm depth. These relationships correlate to water balance in the soil and soil saturation with water. Figure 4 expresses the manifestation of summer rainfall in June and July. The impact of rainfall was also significant in March and September, suggesting their consistency in the form of rain. Precipitation events also increases air humidity. The second factor describes the impact of the precipitation profile and water on the soil. Summer atmospheric rainfall in June and July plays an important role in restoring groundwater reserves for the summer. Summer rainfall during this period is reflected by storm events. The most significant storm events occurred on 23.7.2017 (60.00 mm), 21.9.2017 (60.20 mm), 18.7.2018 (88.40 mm) and 22.7.2018 (69.80 mm).

The third factor, with a variance of 10.595%, is the phenomenon of seasonal contrast of the water cycle in air and soil (Fig. 5). In the winter and autumn months, the air humidity is relatively higher, which in autumn is reflected in the moisture of the leaves along with the occurrence of atmospheric precipitation. The peculiarity of this cycle is the decrease in soil moisture despite the occurrence of atmospheric precipitation. In the winter, precipitation occurs in the form of snow (though in relatively high volumes); soil moisture decreases significantly and the importance of evapotranspiration diminished during this season. The opposite course occurs in April when the spring period begins; hours of daylight increase, wind speed increases, and snow melts. Snow melting increases soil moisture and the change in the phenological phase of plant development causes growth and increase in the importance of evapotranspiration in April. In May, the air humidity increases, resulting in storms and associated rainfall. Plants cease to transpire, but the importance

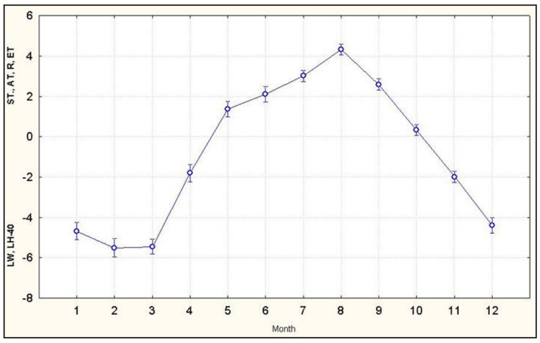

Fig. 3. Factor 1 (F(11,460)=428.55; p=0.0000); LW - lysimeter weight; LH-40 - lysimeter soil humidity in 40 cm; ST - soil temperature (all location); AT - air temperature; R - radiation; ET - evaporation measured by vantage pro 2.

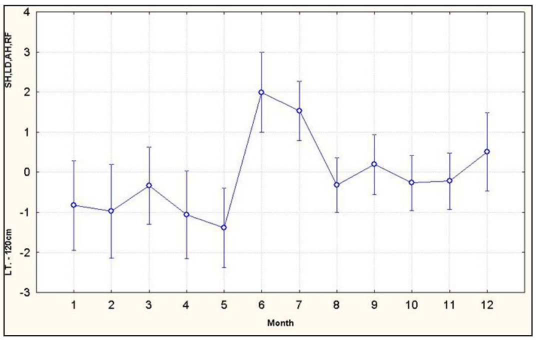

Fig. 4. Factor 2 (F(11.460)=4.6853; p=.00000) LT–120 cm - lysimeter tension in 120 cm; SH - soil humidity (all location); LD - lysimeter drain; AH - air humidity; RF - rain forest (precipitation in forest).

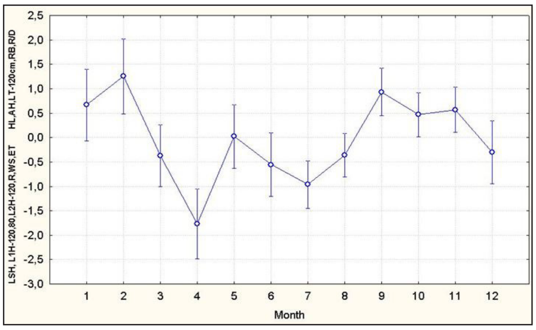

Fig. 5. Factor 3 (F(11.460)=7.5974; p=.00000) LSH - lysimeter soil humidity; L1H-120,80 - locality 1 soil humidity in 120 and 80 cm; L2H - locality 2 soil humidity in 120 cm; R - radiation; WD - wind speed; ET - evaporation measured by vantage pro 2; HL humidity leaf; AH - air humidity; LT-120 - lysimeter tension in 120 cm; RB - rain building (precipitation); R/D – rain/drain.

of interception increases due to the phenological phase of the stand and a small amount of rainfall in the stand, resulting in leaf moisture and soil moisture reduction. Evapotranspiration was likely suppressed due to low rainfall in the stand, causing vegetation to close off vents, while aerial parts of the plants trapped precipitation that evaporated from their surface. In 2017 and 2018, this interception was measured at 38% and 32.8% respectively. In the period between April and August, the Javorová valley forest played a significant role in capturing water, depending on the phenological phase of the stand.

The fourth factor -spring rains- represented a variance of 5.602%. This phenomenon has the most significant effect between March and May (Fig. 6). During this period, the occurrence of spring rains is increased by spring winds. The change in the phenological phase of vegetation in this period, together with spring atmospheric precipitation, changed the character of water vapor to air. In the autumn and winter, evaporation of water into the air is carried out by evaporation, whereas in the spring it is mainly a by product of the transpiration ability of vegetation. Transpiration by vegetation is weak or non-existent during winter and autumn. The spring is characterized by high consumption of atmospheric precipitation by vegetation. The increase in water consumption is due to a change in the phenological phase and the onset of photosynthesis. The progression of this phenomenon is the most significant for coastal growth due to the ability to transpire water in the spring. Long term, these phenomenon and their impacts may be influenced by climate change.

The fifth factor represents a variance of 4.222% and relates to the phenomenon of physical forest interception and the moisture of upper soil horizons. The stand captures the most atmospheric precipitation in June (Fig. 7). At the same time, the soil moisture is comparatively lower in the upper horizons at this time. However, the opposite is true in lysimeter. This can be caused by the relatively small and closed space of the cylinder in the lysimeter, along with the location of the lysimeter next to the stand. In June a lesser amount of rainfall reaches the forest floor due to tree crows. The importance of evaporation from the soil, and water leakage through the soil profile were reduced and the tension of the soil at a depth of 120 cm increased. April, August, September and October measurements showed higher soil moisture, water leakage through the soil horizon, increased evaporation and an increase in the amount of rainfall penetrating through the crown floor. The end of summer through autumn is characterized by lower interception, higher water leakage in the soil and greater environmental evaporation. The summer months, and most significantly the month of June, are characterized by the interception ability of the stand and the decrease in surface moisture.

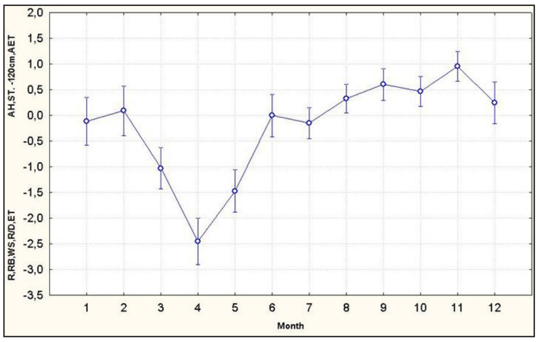

Fig. 6. Factor 4 (F(11.460)=23.741; p=0.0000) R - radiation; RB - rain building (precipitation); WS - wind speed; R/D - rain/drain; ET - evaporation measured by vantage pro 2; AH - air humidity; ST-120 - soil temperature in 120 cm (locality 1 and 2); AET - actual evaporation calculated from lysimeter weight.

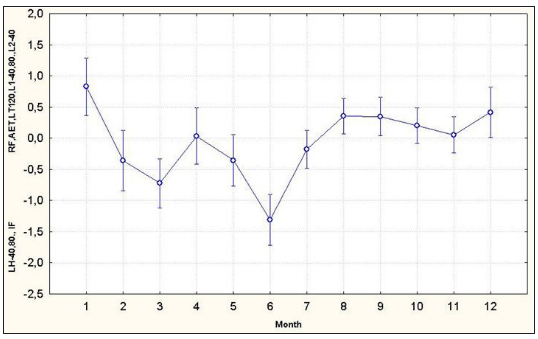

Fig. 7. Factor 5 (F(11.460)=8.0009; p=.00000) LH-40,80 - lysimeter soil humidity in 40 and 80 cm; IF - interception forest; RF - rain forest (precipitation in forest); AET - actual evaporation calculated from lysimeter weight; LT-120 - lysimeter tension in 120 cm; L1 -40,80 - Locality 1 soil humidity in 40 and 80 cm; L2-40 - locality 2 soil humidity in 40 cm.

Course of chemical components in the season

The variability of sulfur values throughout the year is very high. In January, February and March, sulfur levels are below the detection limit, with an occasional exception for water samples from 120 cm, 40 cm, and wells. Between April and August, sulfur levels were at their highest concentration. From September to December, sulfur values were often below the detection limit in well water samples. It follows that the highest values during the season were measured in June, July, August and the lowest values occurred in spring, autumn and winter (Fig. 8a). The most stable pattern occurred in a well water control sample where the water level was less than 120 cm during the year.

The seasonal profile of potassium in the soil monolith, along with the well water control sample and drainage water, followed the same pattern of variance (Fig. 8b). The lowest measured values occurred in the months of November, December, January and February. The highest values were measured during March and October, with the exception of July. The cycle of potassium values oscillated during the year, depending on seasonal changes.

Rubidium distribution was not significantly dependent on the season, although in some cases (for example March - Rb 80, Rb 120, Rb well) it appeared to be affected by seasonal characteristics (Fig. 8c). The upper part of the soil monolith (Rb 40) exhibited relatively similar rubidium concentrations from January to May with a deviation from this standard occurring in February. Increased values persisted until the end of the year with oscillations to lower values in August and November. The middle part of the soil monolith (Rb 80) exhibited a significantly different rubidium profile when compared to the upper soil monolith. Lower values were measured early in the year (January, February) as well as at the end of the calendar year (November, December) as well as in May. Between March and August, there were changes rubidium concentration (Fig. 8c) with the highest values occurring in March and July. September and October were characterized by moderation and stabilization of changes in values. Lower parts of the soil monolith (Rb 120) had the most significant increase of values in March. Prior

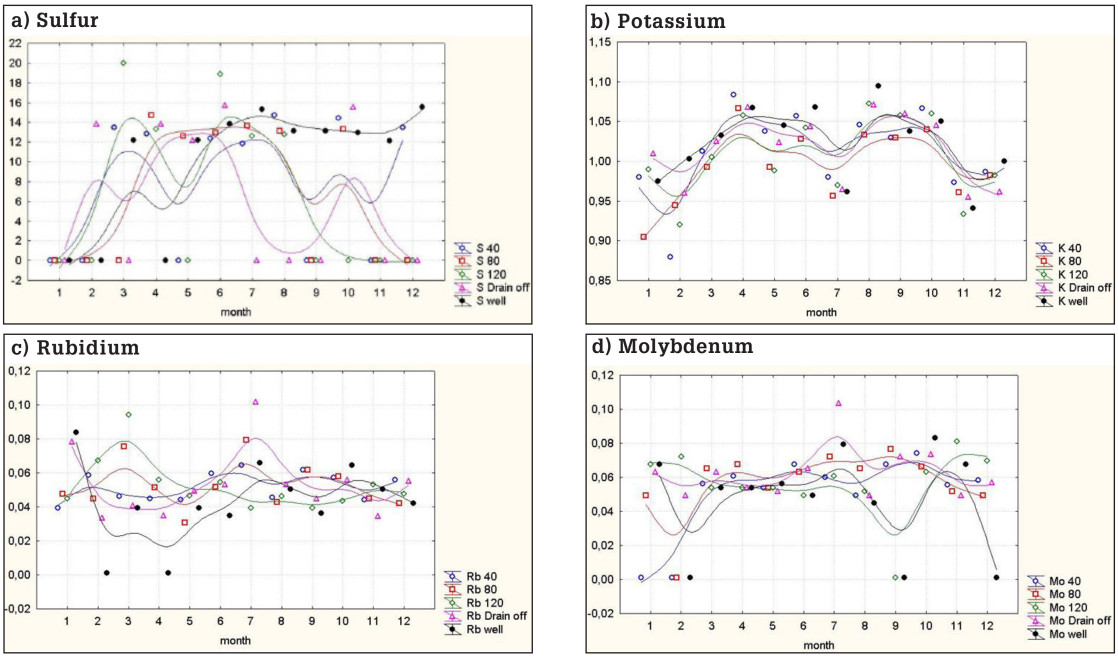

Fig. 8. Profile of chemical components throughout the year.

to March, concentrations of rubidium generally declined, and then increased again near year-end. The values of rubidium in drainage and well water followed the same pattern. The highest measured value of rubidium was taken in the month of June in a water sample from drain-off.

Molybdenum (Fig. 8d) concentrations show a period of high variability of values between September and February; a period of low variability between March and May; and a period of increased variability and increase in values between June and August. The high variability period (September to February) occurs due to low concentrations of molybdenum in September and again in February. In March, molybdenum levels stabilized and subsequent homogeneity of values persisted until May. In May, values of molybdenum were relatively equal in all monitored locations. In June, variability increased again particularly in the upper (40 cm) and middle layers (80 cm) as well as in the lysimeter reading. The opposite pattern emerged for the upper layers (120 cm) and for well samples. In July, there was an increase in molybdenum content in almost every monitored location with the exception of the upper soil monolith, where the value decreased. In August, there was a decrease in molybdenum concentrations across all locations.

Discussion

Antal *et al.* (2003) consider soil moisture to be a fundamental characteristic of water content in soil. Water in soil is the most important factor affecting the water cycle regime (Tužinský 1993). Soil moisture at individual locations during the monitoring period varied depending on the season. An exception occurred in the lysimeter measurements when the soil moisture was relatively constant. We can infer that this occurred as a result of the design of the lysimeter system as it creates a closed soil system that prevents the natural distribution of water in the soil when compared to the forest ecosystem. The highest variability was recorded from soil probes at site 1 and 2. We anticipated that the highest soil water content would occur during snow melt, when groundwater reserves are replenished. Tužinský (1993) predicted a favorable soil moisture content by June, assuming sufficient precipitation in winter and spring. However, in the spring, the soil moisture values were at their lowest levels, which did not confirm the Tužinský supposition. Lapin *et al.* (1990) predicted an increase in potential evapotranspiration of 7-14% by the year 2000, and an increase up to 18% by the year 2020, which would have the effect of drastically reducing runoff and increasing water deficiency. Tužinský (1993) hypothesized that evapotranspiration would consume the winter water supply in early spring and subsequent evapotranspiration would be forced from May to September. Consumption of winter water supply by transpiration (Tužinský 1993) was confirmed.

The principal component analysis was used to evaluate the factors influencing the soil moisture regime of the monitored stand. The first factor presented the contrast in evapotranspiration in summer and winter. The effect of evapotranspiration in Javorová valley was the most significant from May to October. Tužinský (1993) predicted the onset of forced evapotranspiration for the period between May and September, based on a change in soil moisture caused by climate change. The most significant manifestation of a evapotranspiration event in the period from May to August confirmed this hypothesis. Šiška *et al.* (2005) evaluated the changes in total potential and actual evapotranspiration as well as the evapotranspiration deficit in Slovakia's elevation profile from the perspective of possible climate development. The results suggest an increase in potential evapotranspiration of 9% by

2010, 15% by 2030; and 25% by 2075 for northern parts of Slovakia (Šiška *et al.* 2005). Changes are also expected to be 6% in 2010, 9% in 2030, 13% in 2075, while the evapotranspiration deficiency could increase by up to 32% by 2010, 54% by 2030 and 111% by 2075 (Šiška *et al.* 2005). Evapotranspiration is considered to be an important component of the water balance and its expected increase due to climate change suggests an increase in longer-term drought, as well as greater and faster evapotranspiration in the future.

The fourth factor showed the impact of spring rain on the vegetation under investigation. We can infer that the of spring rain (from March to May) was probably influenced by the phenological phase of the stand. Škvareninová (2009) reported a period of budding of needles (*Picea abies*) for stands above 950 meters in the second half of April.

In forest ecosystems, loss of water balance through interception is influenced by stagnation, canopy, woody composition and age of vegetation (Tužinský 2004). The impact of physical forest interception on the moisture content of top soil horizons was most significant in June. In in June of 2017, interception accounted for 73.5 mm or 54.2% of rainfall out of a total 135.6 mm that fell in the open area. In 2018, precipitation was significant in June, and 244 mm rainfall fell in the open area. During this time period, interception captured 64.2 mm or 26.3%. According to Tužinský (1997), the process begins with the penetration of rainfall through the crown floor from ≥1.0 mm. The percentage of rainfall penetration varies depending on the foliage stage. Results show that the amount of water trapped by interception depends on the amount of precipitation.

The second factor measured was important in terms of water balance components and describes the profile of rainfall, water and soil impact. The most significant relationship occurred between rainfall and soil moisture. Impact collisions during storm events occurred in June and July. At higher altitudes and areas predisposed to fog, horizontal rainfall (Fojt and Krečmer 1975) contributes significantly to water totals. The effect of horizontal rainfall on the water balance of the stand is an increase in the amount of water leaked through the soil monolith, and increase in soil and air humidity. These effects were most significant in June and July. Rainfall during this period was high in volume and intensity, but there was a prolonged time period between rainfall events. When the stand had sufficient water content, these rainfall events replenished the groundwater levers. Forest stands play an important role in the hydric function of the ecosystem (Lepeška 2008).

The third factor for analysis is the effect of seasonal changes and characteristics on water balance. Holko *et al.* (2011) lists specific characteristics of the hydrological cycle in mountain environments, including: distribution of basic climatic elements, vegetation, height differences and the impact of complex landscape morphology. The water cycle influences the soil moisture regime. In the winter, the surface of soil freezes, snowfall increases air humidity, and soil moisture decreases. Change in moisture conditions correlate with the change of season and the phenological phase of the stand. Tužinský (2006) reports solar radiation as the most important factor affecting phenological phenomena. The peculiarity of this cycle was the decrease of soil moisture despite the occurrence of atmospheric precipitation. From a hydropedological point of view, a significant factor is the depth of the soil to which the roots reach, which, through deduction, affects the water supply in the soil (Tužinský 2006). These factors, along with the climatic elements, landscape morphology, phenological phase of the stand and the depth of the soil, influence the soil moisture regime.

Measurements of element concentrations showed high sulfur variability during the year as it undergoes various photochemical, oxidative, catalytic and other reactions. Sulphur is one of the most common air contaminants, entering the environment through the burning of fossil fuels, volcanic activity, as well as though the biological processes of soil microorganisms (Prousek 1991). Variation in sulphur concentrations in Javorová valley depend both on climatic conditions and the chemical composition of precipitation. Changes to the chemical composition of precipitation is more likely to occur in June, July, and August when sulfur levels are highest. Soil acidification by sulfur was the most significant in the upper soil layers and in the well water control sample. We observed that acidic deposition by rainfall has the greatest impact on groundwater reserves.

The seasonal cycle of potassium exhibited the same type of variation in all the monitored components. The lowest values were recorded in November, December, January, and February and highest values were observed between March and October. In July potassium levels was significantly lower, comparable to winter values. Acid rain in mountain environments leaches calcium, magnesium, potassium and sodium from the soil (Hruška *et al.* 2009). From the measurements, we assume that the lower potassium content in the soil components is due to the higher sulfur content. Potassium belongs to the biogenic elements and its concentration in cellular fluids plays an important role in healthy organism development. Its function is partly the regulation of the plant's water regime. Because it is significantly osmotic, potassium increases the hydrophilicity of the protoplasm, increases osmotic pressure, and reduces evaporation. According to Ložek (2000), potassium content in soil is primarily inorganic potassium with a small proportion of organic potassium biologically absorbed from dead plant tissues and soil microflora. It is likely that potassium activates enzymes that cause protein synthesis (Procházka and Macháčková 1998). Potassium plays a major role in plant health. Potassium leaching is a negative effect of long-term soil acidification. According to Michalík (2001), potassium deficiency causes anion and carbohydrate accumulation in plant tissues, decreases the intensity of biochemical processes, putrescine formation, chlorosis, necrosis and leaf wilt. Potassium deficiency could cause a number of problems in the future, and its deficit will contribute to disrupting the ecological stability of ecosystems.

Rubidium concentration was not significantly dependent on the season, although in some cases it could be affected by seasonal characteristics. In nature, free rubidium does not occur, except in compounds in which the oxidation stage I exits as a Rb cation. Kabata-Pendias (2000) included rubidium in a group of elements with a low degree of potential threat. The values of this element were balanced over the course of the year. Minerals containing rubidium may cause a higher concentration of this element in water. Some plants respond to potassium deficiency by starting to absorb rubidium. One or more of these reasons may have caused this element to decline or increase. Although we are not aware of a biological imperative for this element, we know that it has a stimulating effect on metabolism, similar to potassium.

We classified the seasonal variability of molybdenum into three periods depending on the nature of element variability. Molybdenum is an oft forgotten microelement, but it is a significant microbiogenic element (Marschner 2002). It is part of more than sixty enzymes catalyzing various oxidation-reduction reactions (Mendel and Schwarz 1991). Molybdenum occurs in natural water in trace amounts, usually below 1 μg.dm^3.

Acknowledgements

We would like to express our thanks to Dr. Andrea Pogányová, prof. Marian Janiga, and everybody who contributed to help us in this study. This study was supported by project ITMS (Grant No. 26110230078).

References

Allen, R.G., Pereira, L.S., Raes, D. and Smith, M. 1998: Crop evapotranspiration-Guidelines for computing crop water requirements. FAO Irrigation and drainage paper 56. FAO, Rome.

Antal, J., Igaz, D. and Špánik, F. 2003: Vplyv meteorologických faktorov na predvegetačnú pôdnu vlhkosť v rôznych pestovatelských systémoch. In: *Seminář „Mikroklima porostu"* (eds. J. Rožnovský J. and T. Litschmann), pp. 15-22., ČHMÚ, Brno.

Boczoń, A., Kowalska, A., Dudzińska, M. and Wróbel, M. 2016: Drought in Polish Forests in 2015. *Pol. J. Environ. Stud.*, **25**: 1857-1862.

Bugala, M. and Migas, M. 2011: Variability of qualitative traits of stems, crowns and health state (damage) of white alder in the Poľana Mts. *Acta Facultatis Forestalis Zvolen*, **53**: 7-18.

Bugala, M. and Parobeková, Z. 2016: Influence of altitudinal gradient on productive potential of grey alder (*Alnus incana* (L.) Moench) in the Bukovské vrchy Mts. *Zprávy lesnického výzkumu*, **61**: 175-180.

Fojt, V. and Krečmer, V. 1975: Tvorba horizontálních srážek z mlhy a jejich množství ve smrkových porostech středoevropské oblasti. *Vodohospodářský časopis*, **23**: 581-606.

Holko, L., Kostka, Z., Danko, M. and Liová, S. 2011: Hydrologický cyklus v horskom prostredí. *Životné prostredie*, **45**: 59-63.

Hruška, J., Oulehle, F., Krám, P. and Skořepová, I. 2009: Účinky kyselého deště na lesní a vodní ekosystémy 2. Vliv depozic síry a dusíku na půdy a lesy. *Živa*, **3**: 141-144.

Kabata-Pendias, A. 2000: Trace elements in soils and plants, fourth. CRC Press, Boca Raton, USA.

Kantor, P. 1983: Výpar z půdy a přízemní vegetace ve smrkovém a bukovém porostu. *Práce VÚLHM*, **63**: 165-199.

Kontriš J., Benčaťová, B., Kontrišová, O. and Benčať, T. 2005: A contribution to the knowledge of the phytocoenoses of the marchland-willow shrubs, floodplain forests and slope alder growths in the Pieniny National Park. *Acta Facultatis Ecologiae*, **13**: 5-9.

Krečmer, V. 1962: Lesnícka meteorologie a klimatologie. In: *Praktická rukověť lesnícka 1* (eds. M. Vyskot *et al.*), pp. 13-34. SZN, Praha.

Lapin, M., Faško, P. and Košťálová, J. 1990: Zhodnotenie zrážkových pomerov na území Slovenska po korekcii systematických chýb meraní zrážok. *Meteorologické zprávy*, **43**: 101-105.

Lepeška, T. 2008: Atribúty krajiny vo vzťahu k jej hydrickým funkciám. In: *Environmentálne aspekty analýzy a hodnotenia krajiny: Identifikácia a stanovenie indikátorov (a indexov) na báze prieskumov krajiny a údajov DPZ* (eds. M. Nováková and M. Sviček), pp. 36-45. Výskumný ústav pôdoznalectva a ochrany pôdy, Slovenská akadémia pôdohospodárskych vied, Bratislava.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. and Wardle, D.A. 2001: Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. *Science's Compass*, **294**: 804-808.

Ložek O. 2000: Racionálna výživa a hnojenie jarného jačmeňa so zreteľom na sladovnícku kvalitu zrna. Jačmeň výroba a zhodnotenie. SPU, Nitra.

Lukáčik, I. and Bugala, M. 2007: Rozbor kvalitatívnych znakov kmeňov, korún a zdravotný stav jelše sivej (*Alnus incana* (L.) (Moench.) a jelše lepkavej (*Alnus glutinosa* (L.) v Laboreckej vrchovine. *Acta Facultatis Forestalis Zvolen*, **2**: 31-41.

Lukáčik, I. and Bugala, M. 2009: Premenlivosť a zdravotný stav (poškodenie) prirodzených populácií jelše lepkavej (*Alnus glutinosa* (L.) Gaertn.) v oblasti Slanských vrchov. *Acta Facultatis Forestalis Zvolen,* **1**: 15-28.

Marschner, H. 2002: Mineral nutrition of higher plants, 2nd ed., Academic Press, New York.

Mendel, R.R. and Schwarz, G. 1991: Molybdoenzymes and molybdenum cofactor in plants. *Crit. Rev. Plant Sci.*, **8**: 33-69.

Michalík, I. 2001: Molekulárne a energetické aspekty príjmu živín v rastlinách. I. vydanie. SPU, Nitra.

Milly, P.C.D., Betancourt, J., Falkenmark, J., Hirsch, R.M., Kundzewicz, Z.W., Lettenmaier, R.J. and Stouffer, D.P. 2008: Stationarity is dead: Whither water management? *Science,* **319**: 573-574.

Minďáš, J., Faško, P. and Kveták, Š. 2010: Lesy Slovenska a voda. TUZ, Zvolen

Novák, V. 1995: Vyparovanie vody v prírode a metódy jeho určovania. Veda, Bratislava.

Pagan, J. 1996: Lesnícka dendrológia. TUZ, Zloven.

Penka, M. 1985: Transpirace a spotřeba vody rostlinami. Academia, Praha.

Penman, H.L. 1948: Natural evaporation from open water, bare soil and grass. *Proc. R. Soc. Lond. A,* **193**:120-146.

Procházka, S. and Macháčková, I. 1998: Fyziologia rastlín. Academie, Praha.

Prousek, J. 1991: Chemické a fotochemické reakce probíhajíci v atmosféře. *Chemické listy*, **10**: 260-263.

Rehák, Š., Janský, L. and Nováková, K. 2006: Fyzikálne procesy v pôde. Fyzika pôdy II. Vydavateľstvo UK, Bratislava.

Smolen, F. and Ostrožlík, M. 1994: Podnebie. In: *Tatranský národný park, Biosférická rezervácia*, (ed. I. Vološčuk), pp. 53-66. Gradus, Martin.

Spectrapure Standards 2015: Certificate of analysis, Reference material for measurement of elements in surface water. SPS-SW2 Batch 127, Norway.

Stradiot, P., Kupec, M. and Rehák, Š. 2014: Spatial differentiation of soil retention and hydraulic properties in Borská nížina Lowland. In: *Extrémy oběhu vody v krajině*. (eds. J., Rožnovský, T. Litschmann, T. Středa and H. Středová), pp. 55, ČHMÚ, Mikulov.

Střelcová, K., Sitková, Z., Kurjak, D. and Kmeť, J. 2011: Stres suchom a lesné porasty, aktuálny stav a výsledky výskumu. TUZ, Zvolen.

Šiška, B., Špánik, F. and Igaz, D. 2005: Vývoj evapotrans-

piácie počas veľkého vegetačného obdobia vo výškovom profile slovenska z pohľadu možného vývoja klímy In: *Seminář „Evaporace a evapotranspirace“*, (eds. J. Rožnovský and T. Litschmann), pp. 81-85. ČHMÚ, Brno.
Škvareninová, J. 2009: priebeh vegetatívnych fenologických fáz autochtónnych populácií smreka obyčajného (*Picea abies* [L.] Karst.) zo Slovenska. *Lesnícky časopis-Forestry Journal*, **55**: 13-27.
Tužinský, L. 1993: Vplyv klimatických zmien na vlhkosť pôdy v lesných ekosystémoch. In: *Agrometeorologická konference 93. Sborník referátů* (eds. T. Litschmann and J. Rožnovský), pp. 44-48. ČBkS, Brno.
Tužinský, L. 1997: Bilancia vody v dubovom ekosystéme vo vegetačnom období. Medzinárodná vedecká konferencia Les - Drevo - Životné prostredie. TUZ, Zvolen.
Tužinský, L. 2004: Vodný režim lesných pôd. TUZ, Zvolen.
Tužinský, L. 2006: Regime and dynamics of soil moisture in forest ecosystems of Zahorská lowland. *J. For. Sci.,* **52**: 108-117.
Tužinský L., 2007: Bioklimatológia. 2. vydanie, TUZ, Zvolen.
Vida, T., Leštianska, A., Škvarenina, J. and Střelcová, K. 2012: Využitie meteorologických požiarnych indexov pri hodnotení rizika vzniku požiaru v oblasti Zvolenskej kotliny a okolia v priebehu klimatický odlišných rokov. *Meteorologický časopis*, **1**: 33-39.
Vološčuk, I., Bohuš, I., Bublinec, E., Bohušová-Hradiská, H., Drdoš, J., Dúbravcová, Z., Gáper, J., Greguš, C., Haková, J., Chovancková, B., Hindák, F., Janiga, M., Kocián, Ľ., Korbel, L., Koreň, M., Kováč, J., Kyselová, Z., Lazebníček, J., Linkeš, V., Ložek, V., Majzlan, O., Marenčák, M., Midriak, R., Nemčok, J., Novák, V., Olejník, J., Ostrožlík, M., Pacl, J., Paclová, L., Schmidt, M., Smolen, F., Spitzkopf, P., Šoltésová, A., Šoltés, R. and Šomšák, L. 1994: Tatranský národný park, Biosférická rezervácia. Gradus, Liptovský Mikuláš.

Received 19 July 2019; accepted 30 October 2019.

Oecologia Montana 2019, **28,** 70-76

Mercury as a risk factor for woody plants in the locations of Veľký Choč and Čierny Váh

S. GREŠÍKOVÁ[1] and H. OLLEROVÁ

Department of environmental enginnering, Faculty of ecology and environmental sciences, T.G. Masaryka 24, Technical university of Zvolen, SK-960 53 Zvolen, Slovak Republic; [1]e-mail: simonagresikova@gmail.com

Abstract. In our work we studied samples of *Abies alba* Mill., *Picea abies* Karst., and forest soils. These species are significant as a bio indicator of pollution. Samples were collected from two locations: Veľký Choč and Čierny Váh. At each locality we selected twelve field sites, from which we collected samples of needles at the end of the growing season. They were analyzed with the Advanced Mercury Analyzer AMA 254 (single-purpose absorption spectrophotometry). Soil samples taken from both localities had mercury concentration values ranging from 0.1474 to 0.15224 mg.kg^{-1}. Concentration in assimilation organs had mercury concentration values ranging from 0.00635 to 0.15224 mg.kg^{-1}. The highest value was measured in three year old needles from Veľký Choč – 0.15224 mg.kg^{-1}. We can confirm that the observed areas are not contaminated by mercury.

Key words: Abies alba, Picea abies, assimilation organs, forest soil

Introduction

Mercury (Hg) is a serious pollutant of all environmental components including the food chain, and its effects are often negative. Originally, mercury contamination was considered an urgent issue local to Slovakia, but it has grown into a worldwide chronic problem. It is one the oldest known metals, and gets into the environment mainly through volcanic activity and the burning of coal. This toxic element is used in industrial applications, agriculture, and medicine. An important geochemical barrier to mercury is the soil in which Hg is suspended, preventing it from contaminating organic matter and other soil components. Under certain conditions, mercury may be re-mobilized and transferred to organisms or other environmental components.

Mercury enters the soil as a result of anthropogenic activities such as mining, fossil fuel combustion, application of agrochemicals (pesticides, fertilizers), liming, sewage sludge and household and industrial waste. Heavy metals are also released the the parent rock in the process of pedogenesis (Naidu *et al.* 1996). The soil under trees contains two to three times more mercury than areas without tree vegetation (Kolka *et al.* 1999). Soils with increased humus contain higher concentrations of Hg. Methyl mercury in soil sediments does not exceed 1.5% of the total mercury content (Cornelis *et al.* 2005).

Soil mercury pollution is affected by several factors including soil moisture, sunlight and temperature (Frescholtz and Gustin 2004; Gustin *et al.* 2004; Gustin and Stamenkovic 2005). The speed of this process depends on the soil composition, the amount of clay minerals, organic matter, pH and redox potential. The mercury content increases in surface horizons. Its volatility increases with alkalinity of the environment and with increasing temperature (Gábriš 1998; Zaujec 1999). According to Vojtáš (2000), mercury accumulates mainly in humus horizons due to its volatility. Mercury in plants is considered to be one of the most toxic elements. It affects their physiological and biochemical reactions with high affinity for SH - groups of amino acids. Plants take up both organic and inorganic forms of Hg through their root system. The roots often contain high concentrations of mercury - up to 95%, but rarely, mercury can be transferred into the stem, where concentrations are less than 5% (Millhollen *et al.* 2006).

The penetration of toxic substances is influenced by several soil factors such as soil pH, soil types, humidity, soil accessibility, temperature, soil humus content, heavy metal concentration and form, oxidation-reduction conditions around the root system and the use of fertilizers and plant protection products. Factors influencing the content of absorbed metal by a plant are the movement of metal from the soil to the surface of the root system, contamination and the form of metal in the soil solution, transport of the metal from the surface parts to the inner parts of the roots and translocation of the metal from the root to shoots (Broadley *et al.* 2007).

Leaves are covered with a thin layer of lipids called cuticula. Its role is to protect the leaf from pollutants and water loss. Concentrations of mercury in leaves correlate positively with leaf age. Leaves accumulate the most mercury during the growing season. The assimilation organs absorb atmospheric Hg through the vents, where they are subsequently converted to Hg_2 (Rea *et al.* 2002). Concentrations of Hg from the leaves and trunk may be washed away by precipitation or may gradually fade from the leaves, as this metal is characterized by a high evaporation rate (Munthe *et al.* 1995; Schwesig and Matzner 2001; St. Louis *et al.* 2001). The bark and leaves turn out to be the largest assimilates of Hg from the atmosphere. Air deposition is responsible for up to 90% of Hg uptake in plants. Tree bark only absorbs 10% of mercury. Mercury affects the photosynthesis in both light and dark phases. Intensity is inhibited by more organic compounds than inorganic compounds (Hronec 1996). The highest concentrations were recorded during high photosynthetic activity. The rate of intake of Hg decreases toward the end of the growing season. Absorption of Hg is influenced by wind speed, morphology (hair, wax) and leaf texture (Lindberg and Stratton 1998; Rea *et al.* 2002). Mercury is not only attached to the leaf surface, but also penetrates the epidermis and plant tissues. It enters the leaves primarily through trichomes, cuticles and dental openings as Hg^0. Plants not only sustain mercury content in their leaves, but also excrete it in gaseous form. This leaf absorption of mercury has been proven in the following plant species: *Acer rubrum*, *Picea abies* and *Liriodendron tulipifera* (Hanson *et al.* 1995). By the end of autumn, deciduous trees can contain up to ten times more mercury (Rea *et al.* 2002). High concentrations of Hg result in complete arrest of the assimilation organ growth (Ericksen *et al.* 2003).

Each type of vegetation represents several aspects of the mercury cycle in forest soils. The overlying organic horizon under coniferous trees accumulates up to 28% more Hg than the organic horizon under deciduous species. In general, conifers are more sensitive to contamination, due to their longevity. Organic material under coniferous vegetation absorbs less UV radiation, and potentially reduces photoreduction and evaporation of Hg (Carpi and Lindberg 1997). The soil under conifers on the south side of the slope accumulates more Hg than the stand exposed on the north side. *Abies balsamea* and *Fagus grandifolia* accumulate more Hg than *Acer* sp., *Larix dexidua* and *Betula* sp. Coniferous tree are excellent bioindicators of environmental pollution, detecting mercury, sulfur, chlorine, fluorine and heavy metal thresholds (Arndt *et al.* 1987). They are characterized by the long life of their needles. Excessive Hg values also cause an increase in mitochondria and endoplasmic reticulum. The limiting ability of photosynthetic CO_2 fixation enzymes has also been demonstrated (Hronec 1996). According to Bielek (2000), mercury intake by plants can be reduced by liming soils or through the use of phosphates, although there are contradictory opinions in this respect.

Mercury binds in RNA, DNA and several types of synthetic polyribosomes. For eukaryotic cells, the organic forms of mercury are toxic CH_3HgCl, C_2H_5HgCl and $Hg(CH_3)_2$ (Liu *et al.* 1992). According to Maňkovská (1996a), Hg $(OH)^2$ and HgOHCl represent the greatest toxicity to plants. Cytoplasm acts as a defence mechanism against toxicity in plants, and has the ability to isolate toxic ions from complexes. The most dominant molecules include glutathione (GSH) and phytochelatin (PCs), which can isolate metal ions from the cytoplasm and subsequently facilitate their transport to the vacuoles. Amino acids (hystidine) and organic acids (citrate) are a natural component of the cytoplasm and act on metal complexes that reduce the toxicity of Hg in plants (McGrath and Zhao 2003).

Mining of mercury was a major industry during the First Czechoslovakian Republic, with maximum production reaching 100 tonnes in 1938. They mined mercury largely from metacinabarite and cinabarite, but also as a by-product from other mining processes and the processing of mineral raw materials. The most famous mercury mineral deposits are Malachov near Banská Bystrica, Merník north of Vranov, Rudňany, the Holy Trinity near Nižná Slana and Zenderling near Gelnica (Zorkovský 1972). River sediments indicate a high mercury content from the Spišsko - gemerské Rudohorie and the surroundings of Banská Bystrica (Maňkovská 1996b; Bodiš and Rapant 1999). Mercury is most often bound to sulfur and appears as complex or simple sulphides. The occurrence of mercury coincides with the mineralization of other metal chalkophilic elements. Mineralization took place in metallogenic regions. Most of the mercury was bound in older formations of siderosulfide phases and sulfide tetraedrites of hydrothermal mineralization in Spišsko - gemerskom Rudohorí. Long-term monitoring of heavy metals shows an improvement in the agricultural production situation.

Concentrations of mercury increase in grain and permanent grassland. In Slovakia, the most contaminated regions are Spišské Nová Ves and Gelnica. This monitoring also points to a gradual decrease in the concentration of mercury in fish and game. In Slovakia, mercury emissions have continued to decrease since 1990, with a reduction of 66.4% in emissions between 2001 and 2014 (Sedlák and Poráčová 2015). The aim of this study is to determine concentrations of mercury in assimilation organs of the tree species *Abies alba,* Mill and *Picea abies,* Karst and in soil near Veľký Choč and Čierny Váh, as well as to perform statistical analysis and evaluation of this data.

Matherial and Methods

Sample assimilation organs (*Abies alba, Picea abies*) and soil were collected from two different remote locations. The first location was the hill of Veľký Choč located at an altitude of 1611 m (GPS coordinates for *Abies alba*: 1. sampling site: E: 19° 20´ 54.08", N: 49° 6´ 27.58"; 2. sampling site: E: 19° 20´ 32.01", N: 49° 6´ 40.16"; 3. sampling site: E: 19° 20´ 43.88", N: 49° 6´ 55.84"; GPS coordinates for *Picea abies*: 1. sampling site: E: 19° 20´ 52.86", N: 49° 6´ 29.07"; 2. sampling site: E: 19° 20´ 28.54", N: 49° 6´ 36.12"; 3. sampling site: E: 19° 20´ 42.52", N: 49° 6´ 54.32"). The second location was Čierny Váh, which has an alititude of 1160 m (GPS coordinates for *Abies alba*: 1. sampling site: E: 19° 52´ 22.73", N: 49° 1´ 28.00"; 2. sampling site: E: 19° 53´ 19.02", N: 49° 1´ 43.08"; 3. sampling site: E: 19° 53´ 58.26", N: 49° 1´ 24.08"; GPS coordinates for *Picea abies*: 1. sampling site: E: 19° 52´ 5.60", N: 49° 1´ 13.44"; 2. sampling site: E: 19° 53´ 15.97", N: 49° 1´ 37.24"; 3. sampling site: E: 19° 53´ 51.69", N: 49° 1´ 33.86". Choč is a dominat peak within the Choč mountains, extending on the northern side of Slovakia, near Ružomberok, Dolný Kubín and Liptovský Mikuláš. Based on its geomorphology, Slovakia belongs to the Alps – Himalayan system and the Carpathians subsystem. West Carpathian province, and Inner West Carpathians subprovince, Fatra – Tatra area, wholes Choč mountains, subassembly Choč, Sielnické vrchy and Prosečné, western part extends into whole Great Fatra, subassembly Šípska Fatra (Mazúr and Lukniš 1978). This area is characterized by a moderate climate, with harsher temperatures found at higher elevations. The average annual temperature is between 4-6° C. Precipitation averages 800-1000 mm per year. July is the hottest month of the year, with average temperatures between 12-16° C. There are 100-150 days of snow cover on average. Soil is comprised dominantly of redzina and cambisol types, and clay, stony soils are the dominant class.

Čierny Váh located in the Turkova nature reserve, which is a significant forested area. Čierny Váh extends on the northen edge of Kráľovohoľských Tatier, which is a part of the Low Tatras National Park protected zone (Majerová 2008). Čierny Váh extends through northen Slovakia, within the Liptovský Mikuláš region. Within Geomorphology, the territory of Slovakia belongs to the Alpine-Himalayan system, Carpathians subsystem, West Carpathian province, Inner West Carpathians subprovince, Fatra – Tatra area, Low Tatras, subassembly Kráľovohoľské Tatry (Mazúr and Lukniš, 1978). Climate conditions in this region depend on the relief and the increasing altitude. This area has a colder climate with more precipitation, averaging 1600 mm. The average annual temperature is between 2-4° C. July is the warmest month of the year. In January, the average temperature is -9° C. There are 130 days of snow cover, on average. Dominant soil types include podzols, rankre, litozeme and cambisol. Loamy sand soils, stony and clay sandy soils are the dominant soil classes (Majerová 2008).

We chose 12 sample locations, from which we collected needle samples at the end of the growing season (September 2018). At each site we collected one twig, which was subsequently divided into one, two, and three year-old needles. The collected samples were placed in polythene bags and transported to the laboratory. Samples were dried at laboratory temperature. Dried *Picea abies* and *Abies alba* needles were homogenized to a fine powder in a FAGOR. Each sample was ground for approximately 3-5 minutes at a frequency of 50 Hz. Samples were measured by an Advanced Mercury Analyser (AMA 254). Soil was dried at laboratory temperature. Dried soil was sifted and samples were measured with the AMA 254. Samples (40-60 mg) were placed into the dosing boat. The first stage of analysis is known as the decomposition phase. During this phase, a sample container with a nominal amount of the matrix is placed inside a pre-packed combustion tube. This combustion tube-heated to ~750° C through an external coil-provides the necessary thermal decomposition of the sample into a gaseous form. The evolved gases are then transported (via an oxygen carrier gas) to the other side of the combustion tube. This portion of the tube, pre-packed with specific catalytic compounds, represents the area in the instrument where all interfering impurities (i.e. ash, moisture, halogens, and minerals) are removed from the evolved gases. Following decomposition, the cleaned, evolved gas is transported to the amalgamator for the collection phase. The amalgamator, a small glass tube containing gold-plated ceramics, collects all of the mercury in the vapor. With a strong affinity for mercury and a significantly lower temperature than the decomposition phase, the amalgamator is capable of trapping all mercury for subsequent detection. When all mercury has ben collected from the evolved gases, the amalgamator is heated to ~900° C essentially releasing all mercury vapor to the detection system. The released mercury vapor is moved through to the final phase of analysis-the detection phase. During the detection phase, all vapor passes through two sections of an apparatus known as a cuvette. The cuvette is positioned in the path length of a standard Atomic Absorption Spectrometer. This Spectrometer uses an element-specific lamp that emits light at a wavelength of 253.7 nm, and a silicon UV diode detector for mercury quantitation.

Data was processed using the STATISTICA 7 statistical program. Element measurements were analysed using analysis of variaton and Duncan´ s test, which determined concentrations of mercury for different ageed needles, locations, and species.

Results

The concentration of mercury in assimilation organs Abies alba

The highest value of the soil sample was measured in *Abies alba* in the locality Veľký Choč at the 3rd sampling site at altitude (818 m a.s.l.) of 0.2309 $mg.kg^{-1}$ (Fig. 1). Measured values in *Abies alba* at locality Veľký Choč increase with altitude while values in the locality of Čierny Váh decrease.

According in the Fig. 2 in the locality Veľký Choč, mercury values are decreasing. Two year old needles had the highest mercury concentrations at 0.02714 $mg.kg^{-1}$ between 3 sampling site at an altitude of 818 m a.s.l.

Mercury levels in samples taken from Čierny Váh are highest in 3 year old needles, and lowest in 1 year old needles. The lowest mercury values were 0.00646 $mg.kg^{-1}$, found in 1 year old needles (Fig. 3) from the 2nd sampling site, with an altitude of 905 m a.s.l. Concetration of mercury in the needles of *Abies alba* was higher at the locality of Veľký Choč than at Čierny Váh, which may be as a result of industry (particularly Mondi SCP operations), highway construction, heavy traffic and long – distance emissions. The assimilation organs of *Abies alba* accumulate less mercury than *Picea abies* needles. Overall, avarege mercury values show that spruce needles accumulate more mercury. According to these localities, the mercury content is higher in the abies in the Veľký Choč than in the spruce in the Čierny Váh

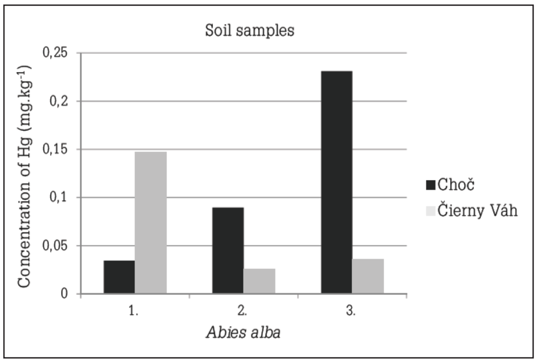

Fig. 1. The concentration of Hg in soil samples near *A. alba* on the investigated sites.

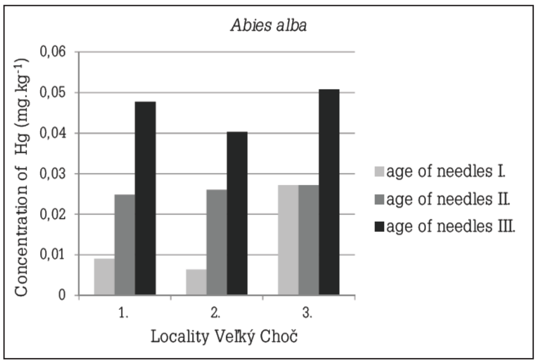

Fig. 2. Concentration of Hg in assimilation organs of the *Abies alba* at locality Veľký Choč.

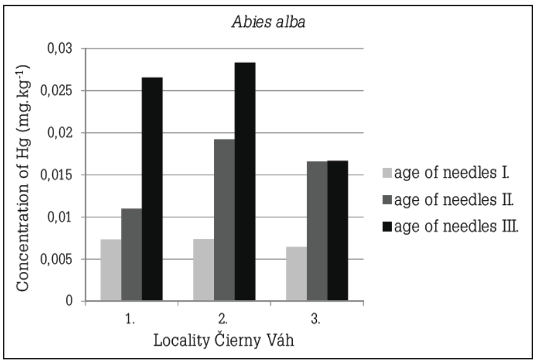

Fig. 3. Concentration of Hg in assimilation organs of the *Abies alba* at locality Čierny Váh.

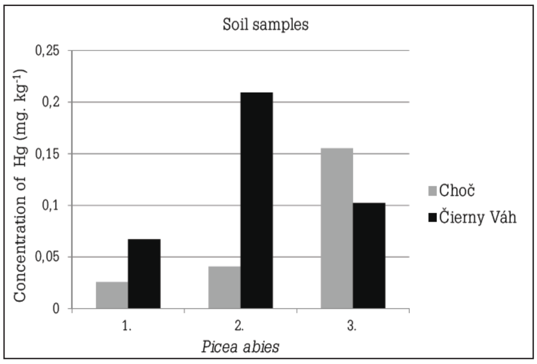

Fig. 4. The concentration of Hg in soil samples near *P. abies* on the investigated sites.

Fig. 5. Concentration of Hg in assimilation organs of the *Picea abies* at locality Veľký Choč.

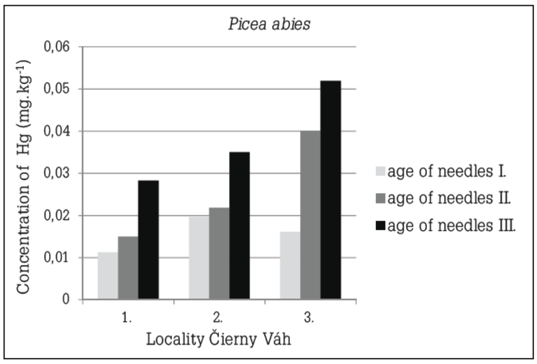

Fig. 7. Concentration of Hg in assimilation organs of the *Picea abies* at locality Čierny Váh.

The concentration of mercury in assimilation organs Picea abies

At the first sampling site, located at an altitude of 751 m a.s.l. in the locality of Čierny Váh, we measured the lowest mercury values of the soil samples (0.06701 mg.kg^{-1}) (Fig. 4).

As per Fig. 5, in the locality of Veľký Choč, mercury values are decreasing. Needles at 3 years of age have the most mercury at 0.01182 mg.kg^{-1}, then 2 year old needles, followed by 1 year old needles with the lowest concentration, measured at 0.01678 mg.kg^{-1}.

Fig. 6 shows the lowest mercury values were measured at Čierny Váh, with a concentration of 0.01122 mg.kg^{-1} for the 1 year old needles, at the 1st sampling site with an altitude of 751 m a.s.l. The highest mercury values were measured in 3 year old needles, at 0.05193 mg.kg^{-1} 3 at an altitude of 1039 m a.s.l.

Statistical processing

Based on the variant analysis, the differences in mean mercury concentrations between *Picea abies* and *Abies alba* are statistically significant. The average value of mercury in spruce needles (0.03085 mg.kg^{-1}) is higher than in *Abies alba* needles (0.02134 mg.kg-1) (Fig. 7).

There is a statistically significant difference in mercury concentration between the locality near Veľký Choč 2. (2nd sampling point) and other locations. The highest average value of mercury was at the 2nd sampling site in Veľký Choč at an altitude of 704 m a.s.l. - 0.0435 mg.kg^{-1} (Fig. 8). The lowest was measured at the locality of Čierny Váh, the 1st sampling point - 0.01683 mg.kg^{-1}. Mercury concentrations show a lesser degree of variance at Čierny Váh than Veľký Choč.

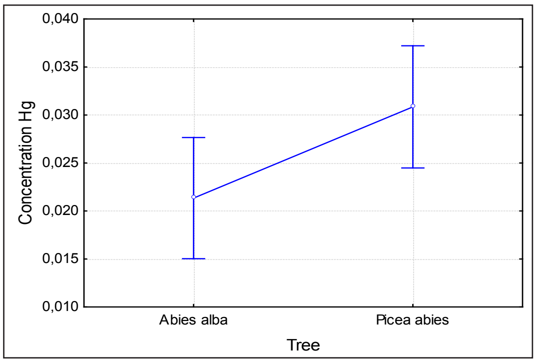

Fig. 8. The differences in mean Hg concentrations between *Picea abies* and *Abies alba* ($F(1, 113)=4.4110$, $p=.03793$; 0.95 confidence intervals).

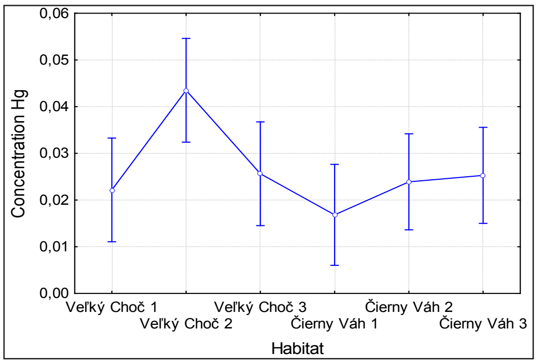

Fig. 9. The difference in Hg concentration between the locality near Veľký Choč and Čierny Váh ($F(5, 109)=2.6479$, $p=.02673$; 0.95 confidence intervals).

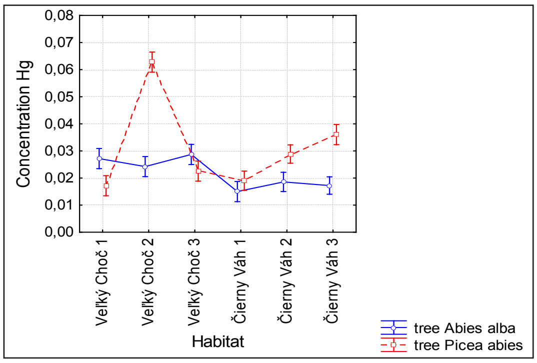

Fig. 10. Comparison of Hg contents between *Abies alba* and *Picea abies* in all sampling sites ($F(5, 79)=46.095$, $p=0.0000$; 0.95 confidence intervals).

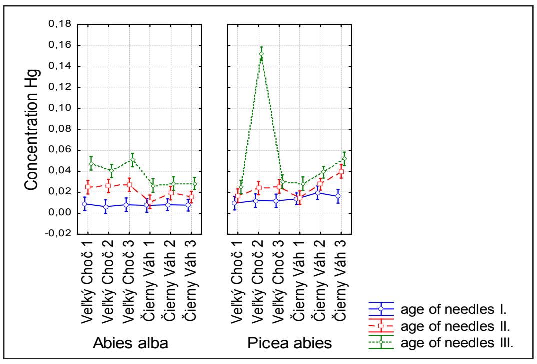

Fig. 11. The mercury concentrations increase with the age of needles.

The mercury contents are lower in *Abies alba* and higher in spruce at the locality Čierny Váh at all three sampling sites. In the locality of Veľký Choč, there are higher mercury values for the first and third sampling point for *Abies alba* as well as the second sampling site for spruce (Fig. 9).

The highest mercury concentrations were found in 3 year old Norwegian spruce (*Piecea abies*) needles at the second sampling site of Veľký Choč (Fig. 10). The values in the first year needles of the *Abies alba* are comparable between both samplig sites while the two-year needles of the *Abies alba* show higher values than those in Veľký Choč. The same applies to the 3 year old fir needles sampled. Annualy, spruce needles show higher mercury values at Čierny Váh, with two-year and three-year needles alternating.

Discussion

In order to assess environmental mercury contamination, we took samples of soil from a depth of 10 - 30 cm from two locations at Veľký Choč and Čierny Váh. In Slovakia, marcury values in soil vary between 0.02 and 0.2 $mg.kg^{-1}$ (Beneš and Pabianová 1987). Ďurža and Khun (2002) measuered mercury concentrations at 0.098 $mg.kg^{-1}$. Steinnes (1997), measured global surface soil concentrations of Hg ranging from 0.003-4.6 $mg.kg^{-1}$. In our study conditions according to Act 220/2004 Coll. on the protection and use of agricultural land, the limit value of mercury for clay is set at 0.75 $mg.kg^{-1}$, 0.50 $mg.kg^{-1}$ for clay soils, and 0.15 $mg.kg^{-1}$ for sandy and clay-sand soils. When we compare the measured values with the given limit, we conclude that the monitored locations did not exceed the limit value. The locations examined are not contaminated with mercury. Real Hg values in soil are likely to be of anthropogenic origin including cross-border emission transfer, the Mondi SCP pulp and paper mill, and the SlovTan facility for the manufacture, treatment, and processing of leather. Mercury concentration in soil in Slovakia was addressed by Maňkovská (1996a). The highest concentration was measured in Rudňany (130μg Hg/g), where in the years 1332 - 1992 mercury, iron, and copper were mined. According to Kabata-Pendias and Pendias (1992), Hg values are low, mostly ranging from 0.05 $mg.kg^{-1}$ to 0.30 $mg.kg^{-1}$. According to Čurlík and Šefčík (1999), contaminated soils in Slovakia are present near Volovských vrchoch (lower and middle Spiš). Increased concetrations of mercury on the soil surface occur through the accumulation of mercury from the subsoil, as well as through contamination by emissions. At higher altitudes in northern and north-western Slovakia, mercury values in blanket humus are significantly increased due to long-range transmission by air pollution. Barančíková (1998) determined that mobility of mercury is dependant on pH. Alloway (1990); Barančíková (1998); and Berghofer *et al.* (1996) report that organic materials in soil have excellent absorption properties that affect the bioavailability of metals; particularly those with a high affinity for organic matter like copper and lead (Passdar 1994). According to measurements by Pavlenda *et al.* (2008) the concentration of mercury in the Ool subsiol of blanket humus varied from 0.024 to 0.831 mg.kg^{-1}. In the Oof subhorizon values of 0.057 to 9.518 mg.kg^{-1} were recorded. Glevaňák (2011) also measured mercury in soil, and the highest mercury values were recorded at the locality of Pod Briou in the Zamagurie region at 0.1185 mg.kg^{-1}. Maliková (2013) detected the highest concentration of mercury at the locality of Klokočov (0.114 mg.kg^{-1}) in area of CHKO Kysuce. Pacherová (2010) measured the highest concentrations of mercury in soil in near Štiavnické vrchy in the locality of Podlužany at 0.137 mg.kg^{-1}. Janotíková (2015) recorded the highest concentration of mercury 0.64096 mg.kg-1 at Hnúšťa. This area is located near former chemical plants for rubber production. Filipiak (2009) recorded values of Hg in the range of 0.061-0.287 mg.kg^{-1} near Brezno. The accumulation of mercury in soil horizons may be related to the absorption of Hg by humic substances and the formation of organomineral complexes. On the other hand, due to its volatility (elemental mercury), organic soil horizons have a higher concentration of mercury than mineral horizons. Fostier *et al.* (2000) found that precipitation passing through the crown layer may have up to 3 times the concentration of mercury compared to free surface precipitation. This phenomenon may be due to the washing of mercury trapped on the tree assimilation organs and the subsequent accumulation in the humus horizon (Rea *at al.* 2000). Plants can accumulate these pollutants substances in their root systems. Since *Abies alba* has a deep root system, we can assume that the accumulation of toxic substances instead takes place through the leaves by aerosols. Maňkovská (1996a) in Slovakia measured 0.1080 mg.kg^{-1} of mercury in the needles of *Abies alba*. Maňkovská (1996) measured the concentration of Hg in the needles of norwegian spruce from Rudňany (1.249-4.402 mg.kg^{-1}). According to Maňkovska (1996) the recommended Hg values for assimilation organs is 0,12 mg.kg^{-1}. Rea (2002) recorded growth concentration of mercury in assimilation organs at the end of the growing season in October (0.0360 – 0.0080 mg.kg^{-1}). At the beginning of the vegetation period in May, the concentrations were significantly lower (0.0035-0.0013 mg.kg^{-1}). In Spiš and also in Horná Nitra, Hg values were measured in assimilation organs of *Fagus sylvatica* at 4.01 mg.kg^{-1} (Maňkovská 1996a; Bodiš and Rapant 1999). According to Eriksen *et al.* (2003) Hg values in plant leaves are a function of time and of mercury concentration in the air and do not depend on the mercury concentration in the soil. Maňkovská (1984) measured concentration of Hg 0.12 mg.kg^{-1}. Near the Biosphere Reserve Poľana, Kontrišová *et al.* (2004) measured mercury concentrations of 0.0373 mg.kg^{-1} in two-year needles of *Picea abies*. At Mlynky in Slovenský Raj, Kontrišová *et. al.* (2004) recorded the lowest values (0.0399mg.kg^{-1}) in one year old needles of *Picea abies*, and the highest values in two year old needles of *Picea abies* (0.0842 mg.kg^{-1}). Kontrišová *et al.* (2004), in the mid-Pohron region Prestavlky recorded average values in one year old needles of *Picea abies* (0.0345 mg.kg^{-1}) and higher values in two year old needles (0.0451 mg.kg^{-1}). In 1996, the values of mercury in needles stabilized at 0.1 mg. kg^{-1} (Maňkovska 1996a). Janotíková (2015) measured the highest concentration of mercury in the locality of Hrachovo at 0.02971 mg.kg^{-1}. Kubinčanová (2014) recorded mercury values of 0.0563 mg.kg^{-1} in second year needles for CHKO Kysuce measured at the locality of Nová Bystrica. Maňkovská (1996a) measured average values of mercury in *Picea abies* needles in Horná Nitra at 0.099 mg.kg^{-1}, in Žiar nad Hronom at 0.076 mg.kg^{-1}, in Bratislava at 0.089 mg.kg^{-1} and in Košice at 0.133 mg.kg^{-1}.

Acknowledgements

I thank to Dr. Haas for her patience, and moral support in writing this article. I would like to thank my parents who supported me throughout the study.

References

Alloway, B.J. 1990: Heavy metals in soils. Thomson Litho, Scotland.

Arndt, U., Nobel, W. and Schweizer, B. 1987: Bioindikatoren. Ulmer Verlag, Stuttgart.

Barančíková, G. 1998: Návrh účelovej kategorizácie pôd SR z hľadiska citlivosti k znečisteniu ťažkými kovmi. *Rostlinná výroba*, **44**: 117-122.

Beneš, S. and Pabianová, J. 1987: Přirozené obsahy distribuce prvků v půdach. VŠZ Praha, Praha.

Bielek, P. 2000: Obsahy prvkov v pôdach a ich vzťah k zdraviu človeka. In: *Pedofórum 2000. Zborník príspevkov* (ed. P. Bielek), pp. 76-86. Výskumný ústav pôdoznalectva a ochrany pôdy, Bratislava.

Berghofer, R., Makovníková, J., Mosbach, J., Wilcke, W. and Zech, W. 1996: Schwermetall und aluminiumrektionen slowakische Boden. Jahresbericht Universität, Bayreuth.

Bodiš, D. and Rapant, S. 1999: Geochemický atlas Slovenskej republiky. Geologická služba SR, Bratislava.

Broadley, M., White, P.J., Hammond, J.P., Zelko, I. and Lux, A. 2007: Zinc in plants. *New Phytoll.*, **173**: 677-702.

Carpi, A. and Lindberg, S.E. 1997: Application of a teflon™ dynamic flux chamber for quantifying soil mercury flux: Tests and results over background soil. *Atmos. Environ.*, **32**: 873-882.

Cornelis, R., Caruso, J., Crews, H. and Heumann, K. (eds.) 2005: Handbook of elemental speciation II: Species in the environment, food, medicine and occupational Health. John Wiley.

Čurlík, J.,and Šefčík, P., 1999: Geochemický atlas Slovenskej republiky. Časť V. Pôdy. MŽP SR, Bratislava.

Ďurža, O. and Khun, M. 2002: Environmentálna geochémia niektorých ťažkých kovov. UK, Bratislava.

Ericksen, J., Gustin, M., Schorran, D., Johnson, D., Lindberg, S. and Coleman, J. 2003: Accumulation of atmospheric mercury in forest foliage. *Atmos. Environ.*, ***37***: 1613-1622.

Filipiak, O. 2009: Zhodnotenie kumulácie železa a ortuti v rastlinách a v pôde v regióne Brezno. Thesis. MTF UBEI STU, Trnava.

Fostier, A.H., Forti, M.C., Guimaraes, J.R.D., Melfi, A.J., Boulet, R., Espirito Santo, C.M. and Krug, F.J. 2000: Mercury fluxes in a natural forested catchment (Serra do Navio, Amapá State, Brazil). *Sci. Total. Environ.*, **260**: 201-211.

Frescholtz, T. and Gustin, M. 2004: Soil and foliar mercury emissions as a function of soil concentration. *Water, Air, Soil Pollut.*, **155**: 223-237.

Gábriš, Ľ. (ed.) 1998: Ochrana a tvorba životného prostredia v poľnohospodárstve. 1. vyd. SPU, Nitra.

Glevaňák, J. 2011: Využitie hrúbkového prírastku drevín na rekonštrukciu zmien obsahu ortuti v oblasti Spišská Magura. Thesis. FEE TU, Zvolen.

Gustin, M.S., Ericksen, J.A., Schorran, D.E., Johnson, D.W., Lindberg, S.E. and Coleman, J.S. 2004: Application of controlled mesocosm for understanding mercury plant-soil-air exchange. *Environ. Sci. Technol*, **38**: 6044-6050.

Gustin, M.S., and Stamenkovic, J. 2005: Effect of watering and soil moisture on mercury emissions from soils. *Biogeochemistry*, **76**: 215-232.

Hanson, P., Linberg, S., Tabberer, T., Owens, J. and Kim, K.H. 1995: Foliar exchange of mercury vapor: evidence for a compensation point. *Water, Air and Soil Pollution*, **80**: 374-281.

Hronec, O. 1996: Exhaláty - pôda - vegetácia. 1. vyd. Prešov a Slovenská poľnohospodárska a potravinárska komora, Bratislava.

Janotíková, M. 2015: Kumulácia ortuti v ihliciach smreka obyčajného a v pôde v oblasti Gemera. Thesis. FEE TU, Zvolen.

Kabata-Pendias, A. and Pendias, H., 1992: Trace elements in soils and plants. CRC Press, London.

Kolka, R., Nater, E.A. nad Grigal, D. and Verry, E.S. 1999: Atmospheric inputs of mercury and organic carbon into a forested upland/bog watershed. *Water, Air, Soil Pollut.*, **113**: 273-294.

Kontrišová, O., Ollerová, H., Marušková, A. and Kontriš, J., 2004: Bioindikácia záťaže ortuťou na vybraných lokalitách v oblasti Slovenského stredohoria a stredného Spiša. In: *VI. Banskoštiavnické dni* (eds. P. Hybler and A. Marušková), pp. 77-81. TU, Zvolen.

Kubinčanová, N. 2014: Zhodnotenie kumulácie ortuti v asimilačných orgánoch smreka obyčajného a v pôde v oblasti CHKO Kysuce. Bachelor thesis. FEE TU, Zvolen.

Lindberg, S.E. and Stratton, W.J. 1998: Atmospheric mercury spectation. Concentrations and behaviour of reactive gaseous mercury: in ambient air. *Environ. Sci. Technol.*, **32**: 49-57.

Liu, Y., Cotgreave, I., Atzori, L. and Grafstrom, R.C. 1992: The mechanism of Hg^{2+} toxicity in cultured human oral fibroblasts: te involvemet pf cellular thiols. *Chem. Biol. Interact.*, **85**: 69-78.

Majerová, M. 2008: Zhodnotenie prírodného potenciálu rekreačnej krajiny v okrese Liptovský Mikuláš. Bachelor thesis. FESRR SPU, Nitra.

Maliková, M. 2013: Environmentálne zaťaženie pohraničných oblastí okresu Čadca ortuťou. Bachelor thesis. FEE TUI, Zvolen.

Maňkovská, B. 1984: The effects of atmospheric emissions from the Krompachy, Nižná Slaná, Rudňany iron-ore-mines on forest vegetation and soil. *Ekológia (ČSSR)*, **3**: 331-344.

Maňkovská, B., 1996a: Mercury concentrations in forest trees from Slovakia. *Water, Air and Soil Pollut.*, **89**: 267-275.

Maňkovská, B. 1996b: Geochemical atlas of Slovakia: Forest biomass. Geologická služba Slovanskej republiky, Bratislava.

Mazúr, E. and Lukniš, M. 1978: Regional geomorphological division of Slovakia. *Geografický časopis*, **2**: 101-124.

Mcgrath, S.P. and Zhao, F.J. 2003: Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotech.*, **14**: 277-282.

Millhollen, A., Gustin, M. and Obrist, D. 2006: Foliar mercury accumulation and exchange for three tree species. *Environ. Sci. Technol.*, **40**: 6001-6006.

Munthe, J., Hulberg, H. and Iverfeldt, A. 1995: Mechanisms of deposition of methylmercury and mercury to coniferous forests. *Water, Air and Soil Pollut.*, **80**: 363-371.

Naidu, R., Kookana, R.S., Oliver, D.P., Rogers, S. and Mclaughlin, M.J. (eds.) 1996: Contaminants and the Soil Environment in the Australasia-Pacific Region: Proceedings of the First Australasia-Pacific Conference on Contaminants and Soil Environment in the Australasia-Pacific Region, Held in Adelaide, Australia, 18–23 February 1996. Springer Science & Business Media, Berlin.

Pacherová, D. 2010: Hodnotenie kumulácie Hg (TOT) v drevinách na tranzektoch v CHKO Štiavnické vrchy. Thesis, FEE TU, Zvolen.

Passdar, D. 1994: Bodenempfindlichkeit Schadstoffe Ostereichische Bodekartierung, Schwermetalle, Wien.

Pavlenda, P., Capuliak, J. and Kajba, M. 2008: Evaluation of mercury concentrations in forest soils of Slovakia on permanent monitoring plots. National Forestry Center. Forest Research Institute, Zvolen.

Rea, A.W., Lindberg, S.E., Scherbatskoy, T. and Keeler, G.J. 2002: Mercury accumulation in foliage over time in two northern mixed-hardwood forests. *Water, Air, and Soil Polluti*, **133**: 49-67.

Sedlák, V. and Poráčová, J. 2015: Environmentálna toxikológia. PU Prešov.

Schwesig, D. and Matzner, E. 2001: Dynamics of mercury and methylmercury in forest floor and runoff of a forested watershed in Central Europe. *Biogeochemistry*, **53**: 181-200.

St. Louis, V.L., Rudd, J.W.M., Kelly, C.A., Beaty, K.G., Flett, R.J. and Roulet, N.T. 2001: Production and loss of methylmercury and loss of total mercury from boreal forest catchment containing different types of wetlands. *Environ. Sci. Technol.*, **30**: 2719-2729.

Steinnes, E. 1997: Mercury. In: Heavy metals in soil, 2nd edition (ed. B.J. Alloway), pp. 245-259. Blackie Academic & Professional Press, London.

Vojtáš, J. 2000: Analýza hygienického stavu pôd na Slovensku a návrh doplnenia kódu bonitovaných pôdno - ekologických jednotiek pre kontaminované pôdy. 1. vyd. Výskumný ústav pôdoznalectva a ochrany pôdy, Bratislava.

Zaujec, A. 1999: Cudzorodé látky a hygiena pôd. 1. vyd. SPU, Nitra.

Zorkovský, V. 1972: Ložiská nerastných surovín a ich vyhľadávanie. Alfa, Bratislava.

Recieved 13 August 2019; accepted 11 November 2019.

Oecologia Montana 2019,
28, 77-80

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

N. KUBJATKOVÁ[1] and M. HAAS

Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic;
[1]e-mail: natushha@gmail.com

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	Significancy	R	p	n	Significancy
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.

Acknowledgements

We would like to thank prof. M. Janiga for his assistance during prepare the results, and to Daniela Dúhová, Bibiána Martinková, Filip Korec, Katarína Pinterová, Martin Lukáň, Martina Lukáňová for their help during fieldwork and with laboratory work. The research was supported by project ITMS (Grant No. 26210120016).

References

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.

Dúhová, D. 2018: Bioindication of zinc accumulation in snow voles (*Chionomys nivalis*) from Tatra Mountains. Bachelor thesis. IHMB, University of Žilina. Tatranská Javorina.

Fargašová, A. 2008: Environmentálna toxikológia a všeobecná ekotoxikológia. Orman, Bratislava.

Gorriz, A., Llacuna, S., Riera, M. and Nadal, J. 1996: Effects of air pollution on hematological and plasma parameters in *Apodemus sylvaticus* and *Mus musculus*. *Arch. Environ. Con. Tox.*, **31**: 153-158.

Gurnell, J. and Flowerdew, J.R. 1990: Live trapping small mammals: a practical guide. *Occasional Publications of the Mammal Society of London*, **3**: 1-39.

Janiga, M., Hrehová, Z. and 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. *Pol. J. Ecol.*, **60**: 611-619.

Janiga, M., Hrehová, Z., Dimitrov, K., Gerasimova, C. and Lovari, S. 2016: Lead levels in the bones of snow voles *Chionomys nivalis* (Martins, 1842) (Rodentia) from European mountains: A comparative study of populations from the Tatra (Slovakia), Vitosha and Rila (Bulgaria). *Acta Zool. Bulg.*, **68**: 291-295.

Kanu, I., Achi, O.K., Ezeronye, O.U. and Anyanwu, E.C. 2006: Seasonal variation in bacterial heavy metal biosorption in water samples from Eziama river near soap and brewery industries and the environmental health implications. *Int. J. Environ. Sci. Te.*, **3**: 95-102.

Long, X.X., Yang, X.E. and Ni, W.Z. 2002: Current status and perspective on phytoremediation of heavy metal polluted soils. *J. Appl. Ecol.*, **13**: 757-762.

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

Maceda-Veiga, A., Figuerola, J., Martínez-Silvestre, A., Viscor, G., Ferrari, N. and Pacheco, M. 2015: Inside the Redbox: Applications of hematology in wildlife monitoring and ecosystem health assessment. *Sci. Total Environ.*, **514**: 322-332.

Martiniaková, M., Omelka, R., Stawarz, R. and Formicki, G. 2012: Accumulation of lead, cadmium, nickel, iron, copper, and zinc in bones of small mammals from polluted areas in Slovakia. *Pol. J. Environ. Stud.*, **21**: 153-158.

Martinková, B. 2018: Effects of accumulation of mercury in snow vole (*Chionomys nivalis*). Bachelor thesis. IHMB, University of Žilina, Tatranská Javorina.

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. *Ecotoxicol. Environ. Safety*, **54**: 176-187.

Milner, J.M., Stien, A., Irvine, J., Albon, S.D., Langvatn, R. and Ropstad, E. 2003: Body condition in Svalbard reindeer and the use of blood parameters as indicators of condition and fitness. *Can. J. Zool.*, **81**: 1566-1577.

Monge, C. and León-Velarde, F. 1991: Physiological adaptation to high altitude: oxygen transport in mammals and birds. *Physiol. Res.*, **71**:1135-1172.

Rogival, D., Scheirs, J., De Coen, W., Verhagen, R. and Blust, R. 2006: Metal blood levels and hematological characteristics in wood mice (*Apodemus sylvaticus* L.) along a metal pollution gradient. *Environ. Toxicol. Chem.*, **25**: 149-157.

Roscales, J.L., Munoz-Arnanz, J., González-Solís, J. and Jiménez, B. 2010: Geographical PCB and DDT patterns in shearwaters (*Calonectris* sp.) breeding across the NE Atlantic and the Mediterranean archipelagos. *Enviro. Sci. Technol.*, **44**: 2328-2334.

Rundel, P.W. and Millar, C.I. 2016: Alpine ecosystems. In: *Ecosystems of California, Vol. 29* (eds. E. Zavaleta and H. Mooney), pp. 613-634, University of California, Berkeley, California.

Serbaji, M.M., Azri, C. and Medhioub, K. 2012: Anthropogenic contributions to heavy metal distributions in the surface and sub-surface sediments of the Northern Coast of Sfax. Tunisia *Int. J. Environ. Res.*, **6**: 613-626.

Shah, K., Khan, F., Rizvi, M. and Sadeeq, U. 2007: Effect of cypermethrin on clinico-haematological parameters in rabbits. *Pak. Vet. J.*, **27**:171-175.

Storz, J.F. and Moriyama, H. 2008: Mechanisms of hemoglobin adaptation to high altitude hypoxia. *High Alt. Med. Biol.*, **9**: 148-157.

Tchounwou P.B., Yedjou C.G., Patlolla A.K. and Sutton D.J. 2012: Heavy metal toxicity and the environment. In: *Molecular, Clinical and Environmental Toxicology, Vol. 101* (ed. A. Luch), pp. 134-164. Springer, Basel.

Tete, N., Afonso, E., Bouguerra, G. and Scheifler, R. 2015: Blood parameters as biomarkers of cadmium and lead exposure and effects in wild wood mice (*Apodemus sylvaticus*) living along a pollution gradient. *Chemosphere*, **138**: 940-946.

Waghmare, T.E., Nayaka, H.B., Banklgi, S.K.C. and Tukappa, A. 2015: Quantitative estimation of heavy metals in river water and their toxicity, hematology, gravimetric, serum and tissue biochemistry effects in albino rats. *World J. Pharm. Res.*, **4**: 1415-1425.

White, R.M. 1949: The role of mountains in the angular-momentum balance of the atmosphere. *J. Meteorol.*, **6**: 353-355.

Wołk, E. and Kozłowski, J. 1989: Changes of body weight and hematological parameters in a fluctuating population of *Apodemus flavicollis*. *Acta Theriol.*, **34**: 439-464.

Zhang, H., Wu, C.X., Chamba, Y. and Ling, Y. 2007: Blood characteristics for high altitude adaptation in Tibetan chickens. *Poult. Sci.*, **86**: 1384-1389.

Received 20 August 2019; accepted 6 December 2019.

Oecologia Montana 2019, **28,** 81-84

Genetic diversity of the West Carpathians golden eagle (*Aquila chrysaetos*)

Z. HREHOVÁ[1], J. KORŇAN[2] and M. JANIGA[1*]

[1]*Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic;* [2]*Administration of the Kysuce Protected Landscape Area, U Tomali 1511, SK-022 01 Čadca, Slovak Republic;* [1*]*e-mail: janiga@uniza.sk*

Abstract. The fragmentation analysis was chosen to examine the genetic diversity of golden eagles from the West Carpathians. Different types of tissues were sampled - blood, egg contents, eggshell and feathers - between May 1984 and July 2009. In total, 66 samples were compared. All loci in the tested samples showed F_{IS} values significantly greater than zero, which indicates an excess of homozygotes. There is a remarkable lower heterozygosity in Slovak, West Carpathian golden eagles than in eagles from Spain or Great Britain.

Key words: Aquila chrysaetos, fragmentation analysis, West Carpathians

Introduction

Aquila chrysaetos from Accipitridae is one of four genera of eagles living in the Slovak Republic. Besides the golden eagle; *A. heliaca*, *A. pomarina* and *Hieraaetus pennatus* are inhabitants of the Slovakian region of the West Carpathians. The golden eagle primarily inhabits mountainous areas above 800 m a.s.l. Typical habitats include old forests with mountain meadows and pastures. In Slovakia, *Aquila chrysaetos* is a protected animal under Act no. 543/2002 of the Law on nature and landscape protection and by regulation of the Ministry of the Environment. Additionally, 24/2003 is included in the category of species of European importance. The golden eagle is included in the Red list of threatened bird species in Slovakia as a "vulnerable species". The breeding population in Slovakia is estimated at 120-140 pairs (Nuhlíčková and Maderič 2010).

The eagle is an apex predator, residing at the top of ecological food webs and the species is very sensitive to environmental changes such as chemical contaminants, food shortages, sex ratio distortion, and habitat destruction. Golden eagles represent one of the most important ecological indicators of environmental health (Chang *et al.* 2008)

Microsatellite analysis is a good method for studying population genetics. In this study, fragmentation analysis was chosen because of the reliability of produced data. MSAT is also a good tool for the detection of genetic relationships and diversity, parentage and kinship, as well as in the study of recent population history due to a high mutation rate. In recent history, there are additional studies available regarding micrasatellite analysis of raptor species, including *Aquila chrysaetos* (e.g. Nesje *et al.* 2000; Martinez-Cruz *et al.* 2002; Busch *et al.* 2005; Bourke and Dawson 2006; Dawnay *et al.* 2009). The multiplex PCR assay has been established for the study of White-tailed eagle (Hailer *et al.* 2005) as well as for *Aquila chrysaetos* (Bieliková *et al.* 2010).

Material and Methods

Sample collection and DNA extraction

For analysis, different types of tissues were sampled - blood, egg contents, eggshell and feathers - between May 1984 and July 2009 (Table 1). The samples originate from the West Carpathians, Slovakia. DNA was extracted from blood, feather and eggshell inner membrane using the QIAGEN DN easy Blood and Tissue Kit according to standard protocol for blood and feathers and the protocol for isolation of total DNA from animal tissues with modifications as described Busch *et al.* (2005) for eggshell membrane.

Fragment analysis and genetic diversity

Microsatellite analyses of AA26 and AA36 loci was performed according to Martinez-Cruz *et al.* (2002) and loci AA04 and AA39 according to Dawnay *et al.* (2008). Forward primers were fluorescently labeled with Cy-5. Fragmentation analyses ran on GenomeLab GeXP (Beckman Coulter). Raw data were analysed by GenomLab software (Beckman Coulter). Scored alleles were analysed by Genepop4 (Rousset 2012). Genetic diversity and differentiation (allele frequency, genotype frequency, real and effective number of allele, observed and expected heterosygosity and Hardy-Weinberg equilibrium) were tested. F_{IS} were calculated as in Weir and Cockerham (1984).

Results and Discussion

All of the examined loci were polymorphic. The mean number of alleles per locus was 8.5. All loci in the tested samples showed F_{IS} values significantly greater than zero, which indicates an excess of homozygotes, possibly arising from inbreeding (Table 1). F_{IS} is a coefficient that indicates the overall deviation from the Hardy-Weinberg expectation for all loci within the populations sampled. Positive F_{IS} values indicate increasing homozygosity conditions in a population, while negative values indicate an excess of heterozygotes (Svoboda *et al.* 1985).

Conversely, positive values of FIS do not necessarily indicate inbreeding. These values can be inflated if genetic subpopulations exist, but are recognized when the samples are taken; this situation is known as the Wahlund effect (Wahlund 1928). Bieliková *et al.* (2010) mentioned similar findings for loci AA04 and AA26 in wild-living populations and loci AA04, AA26 and AA36 for captive-bred populations. The value of observed heterozygosity (H0) calculated for each locus is higher (AA26 loci, aa36, AA04, AA39) than the expected heterozygosity (HE). The resulting average value of H0 is clearly lower than HE. Therefore, it can be concluded that the overall genetic variability in Slovak populations of *Aquila chrysaetos* is not sufficient. Consequently, the population has likely exhibited the Wahlund effect.

More details about observed alleles, their frequencies and F_{IS}, as well as number of homozygotes and heterozygotes are shown in Table 2.

Locus	Primer sequences 5´-3´	Repeat motif	Size range (bp)	n	A	Observed allele (bp)	H_0	H_E	F_{IS}
Aa04	F: Cy5-TGCAGCTCAAAAGCAAAGG R: CAACCCCAACTCTCACACCT	$(GT)_{12}$	122-170	45	12	122, 124, 126, 128, 142, 146, 150, 152, 156, 158, 162, 166	0.69**	0.89	0.207
Aa26	F: Cy5-TGCAGCTCAAAAGCAAAGG R: CAACCCCAACTCTCACACCT	$(AC)_{14}$	140-154	66	6	140, 142, 148, 150, 152, 154	0.58*	0.75	0.235
Aa36	F: Cy5-GCAAAGGTAAACTGCATCTGG R: ATGCACTATTGGTAAACAGGCA	$(AC)_{16}$	96-124	66	6	96, 98, 100, 102, 104, 124	0.38*	0.66	0.428
Aa39	F: Cy5-ACAGGCCAGCACCAAGAG R: TTTGGAGCCATTGTTACCGT	$(AC)_{13}$	187-229	51	10	187, 189, 191, 195, 197, 199, 205, 209, 211, 229	0.53*	0.84	0.391

Table 1. Characterization of 4 polymorphic microsatellite loci for the golden eagle *Aquila chrysaetos* (Accipitridae, Aves). n - number of unrelated tested Golden eagle individuals from a Slovak population; A - number of alleles per locus; H0 - observed heterozygosity; HE, - expected heterozygosity; * P = 0; ** P = 0,027.

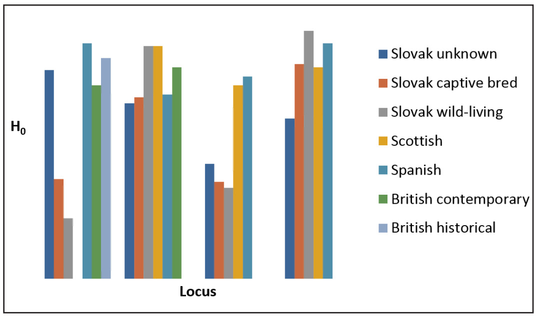

Fig. 1. Comparison of observed heterozygosity among Slovak population of *A. chrysaetos* (this study), captive-bred and wild living eagles (Bielikova *et al.* 2010), and golden eagle population from Scotland (Bourke and Dawson 2006), Spain (Martinez-Cruz *et al.* 2002) and Great Britain (Bourke *et al.* 2010) - loci AA04, AA26. AA36 and AA39, respectively.

In the past, analysis was completed on the populations of family Accipitridae. This includes golden eagle populations from Spain (Martinez-Cruz *et al.* 2002), Scotland (Bourke and Dawson 2006), both the Slovak wild population and individuals bred in captivity (Bieliková *et al.* 2010) and the British contemporary and historical populations (Bourke *et al.* 2010). A graphic comparison of observed heterozygosity among European eagle populations is shown in Fig. 1. There is a remarkable decrease of H0 in wild-living Slovak individuals (0.2) compared to those in captivity (0.33), as well as compared to those of an unknown population from the Slovak republic (0.69) and an additional Spanish individual (0.78).

Acknowledgements

The research was supported by project ITMS (Grant No. 26210120006).

References

Bieliková, M., Ficek, A., Valková, D. and Turňa, J. 2010: Multiplex PCR amplification of 13 microsatellite loci for *Aquila chrysaetos* in forensic applications. *Biologia*, **65**: 1081-1088.

Bourke, B.P. and Dawson, D.A. 2006: Fifteen microsatellite

Locus	Allele size (bp)	Sample count	Freqency	FIS	Homs	Hets
AA04	122	3	0.0333	-0.0233	0	3
	124	3	0.0333	-0.0233	0	3
	126	21	0.2333	0.2033	4	13
	128	20	0.2222	0.3699	5	10
	142	5	0.0566	0.3744	1	3
	146	7	0.0778	0.2361	0	7
	150	3	0.0333	-0.0233	0	3
	152	11	0.1222	0.2854	0	11
	156	7	0.0778	-0.0732	0	7
	158	5	0.0556	-0.0476	0	5
	162	1	0.0111	0.0000	0	1
	166	4	0.0444	0.4854	1	2
	Total	90	0.2074	0.1429		
AA26	140	6	0.0455	0.3085	1	4
	142	48	0.3636	0.0255	9	30
	148	16	0.1212	0.7192	6	4
	150	40	0.3030	0.2182	9	22
	152	10	0.0758	0.3575	2	6
	154	12	0.0909	0.0909	1	10
	Total	132	0.2346	0.3050		
AA36	96	10	0.0758	0.3575	2	6
	98	72	0.5455	0.5167	28	16
	100	20	0.1515	0.6509	7	6
	102	4	0.0303	0.4902	1	3
	104	13	0.0985	0.2393	2	9
	124	12	0.0909	0.0909	1	10
	126	1	0.2074	0.1429	0	1
	Total	132	0.4278	0.3188		
AA39	187	17	0.1667	0.5132	5	7
	189	26	0.2549	0.5935	9	8
	191	25	0.2451	0.4253	7	11
	195	10	0.0980	-0.0989	0	8
	197	3	0.0294	0.6622	1	1
	199	5	0.0490	-0.0417	0	3
	205	5	0.0490	0.3776	0	5
	209	7	0.0686	0.2424	1	3
	211	3	0.0294	0.6622	1	2
	229	1	0.0098	0.0000	0	1
	Total	102	0.3914	0.3309		

Table 2. Observed allele frequencies for 4 polymorphic loci for Slovak population of *Aquila chrysaetos*; Hets – heterozygots; Homs – homozygotes.

loci characterized in the golden eagle *Aquila chrysaetos* (Accipitridae, Aves). *Mol. Ecol. Notes*, **6**:1047-1050.

Bourke, B.P., Frantz, A.C., Lavers, CH.P., Davison, A., Dawson, D. and Burke, T.E. 2010: Genetic signatures of population change in the British golden eagle (*Aquila chrysaetos*). *Conserv. Genetics*, **11**:1837-1846.

Busch, J.D., Katzner, T.E., Bragin, E. and Keim, P. 2005: Tetranucleotide microsatellites for Aquila and Haliaeetus eagles. *Mol. Ecol. Notes*, **5**: 39-41.

Dawnay, N., Ogden, R., Wetton, J.H., Thorpe, R. S. and McEwing, R. 2009: Genetic data from 28 STR loci for forensic individual identification and parentage analyses in 6 bird of prey species. *Forensic Sci. Int. Genet.*, **3**: 63–69.

Hailer, F., Gautschi, B. and Helander, B. 2005: Development and multiplex PCR amplification of novel microsatellite markers in the White-tailed Sea Eagle, *Haliaeetus albicilla* (Aves: Falconiformes, Accipitridae). *Mol. Ecol. Notes*, **5**: 938-940.

Chang, H.W., Chou, T.C., Gu, D.L., Cheng, C.A., Chang, C.C., Yao, C.T., Chuang, L.Y., Wen, C.H., Chou, Y.C., Tan, K.Y. and Cheng, C.C. 2008: An improved PCR method for gender identification of eagles. *Mol. Cell. Probes*, **22**: 184-188.

Martinez-Cruz, B., David, V.A., Godoy, J.A., Negro, J.J., O'Brien, S.J. and Johnson, W.E. 2002: Eighteen polymorphic microsatellite markers for the highly endangered Spanish imperial eagle *Aquila adalberti* and related species. *Mol. Ecol. Notes*, **2**: 323-326.

Nesje, M., Røed, K.H., Lifjeld, J.T., Lindberg, P. and Steen, O.F. 2000: Genetic relationship in the peregrine falcon (*Falco peregrinus*) analysed by microsatellite DNA markers. *Mol. Ecol.*, **9**: 53-60.

Nuhlíčková, S. and Maderič, B. 2010: Raptors and owls of protected bird area Low Tatras (in Slovak). Raptor Protection of Slovakia (RPS), Bratislava.

Rousset, F. 2012: Genepop 4.1.3. Online: http://kimura.univ-montp2.fr/~rousset/Genepop.pdf (retrieved 31.8.2018).

Svoboda, P.L., Choate, J.R. and Chesser, R.K. 1985: Genetic relationships among southwestern populations of the Brazilian free-tailed bat. *J. Mammal.*, **66**: 444-450.

Wahlund, S. 1928: Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehreaus Betrachtet. *Hereditas*, **11**: 65-106.

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, **38**: 1358-1370.

Received 27 September 2019; accepted 10 December 2019.

Oecologia Montana 2019,
28, 85-99

REPORTS

Birds of the mountain range of the Low Tatra National Park, Brankov – Červená Magura, the West Carpathians, 1984 - 1991

M. JANIGA[1] and F. KOREC

Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic;
[1]*e-mail: janiga@uniza.sk*

Abstract. Between 1984 and 1991, the limestone hilly range of Brankov - Červená Magura contained a mosaic of habitats that maintained a rich bird fauna. The European silver fir and beech mixed forest harboured a high number of forest bird species. Above 1000 m, meadows and pastures contained fewer species with lower diversity. Forest edges yielded a higher diversity than that found in typical coniferous spruce forests. Forest edges and mountain meadows harboured a high number of species restricted to these habitat types.

Key words: avifauna of the Low Tatras, Brankov - Červená Magura, the West Carpathians

Introduction

The following account is a summary of our knowledge of the birds of the Brankov - Červená Magura range between 1984 and 1991. In this study area, habitats varied from those characteristic near the artificial tree line, to those of the different types of the forest - from spruce monocultures, to mixed fir, to beech old wood. Because of the variety of habitats, many species of birds were present.

The relationship between bird populaitons and their breeding habitats has interested avian ecologists for quite some time, and much work has been done (Johnston and Odum 1956; MacArthur 1965; Karr 1971; Payne 1982; Moskát *et al.* 1988; etc.) correlating the foliage complexicity of the habitat with bird species diversity.

To date, no information has been gathered as to how habitat pattern relates to breeding bird abundance in Brankov – Červená Magura range in the Low Tatra mountains. Moreover, no information has been found regarding how differences in local weather and climate between years affects bird nesting and behaviour.

This study provides a list of birds living in this limestone dominated part of the Low Tatra mountains and can be used to manage non-game bird populations in the core area of the national park. Data concerning the number of laid eggs, hatchlings and fledglings is presented for some species.

Material and methods

Birds breeding in the different habitats were sampled between mid-April and mid-July, from 1984 to 1991, when the presence of singing or visually observed birds was recorded 5 - 8 times per year in 50 point counts of approximately 20 minutes each.

Results and Discussion

Charadriiformes and Coraciiformes (Fig.1)

Woodcock (*Scolopax rusticola*) - rare but resident, most often seen in unmistakable territorial flight. Roller (*Corracias garrulus*) - vagrant birds observed in autumn.

Galliformes (Fig.2)

Hazel grouse (*Tetrastes bonasia*) lives in mixed woodland or in spruce forest, usually with a dense shrub layer, often near rocks. Black grouse (*Lyrurus tetrix*) - males often seen at open communal diplay grounds. Capercaillie (*Tetrao urogallus*) - rare, but seen, including females.

Accipitriformes (Figs. 3,4) and Falconiformes (Fig.5)

Golden eagle (*Aquila chrysaetos*) young birds were often seen in treeless habitats, and meadows of Veľký Brankov serve as their hunting area. The same is the case for Buzzard (*Buteo buteo*); the species breeds in spruce forests. Sparrowhawks (*Accipiter nisus*) and goshawks (*Accipiter gentilis*) - were observed in suitable hunting areas. Hobby (*Falco subbuteo*) - were rarely recorded, especially in the centre of the mountain range. Kestrel (*Falco tinnunculus*) - the tendancy for the local population of this species to show an increase in size is well known. Birds nest on rock-ledges.

Columbiformes (Fig. 6) and Cuculiformes (Fig.7)

Woodpigeon (*Columba palumbus*) - the mixed beech and spruce forest is a very suitable habitat for this species, as it nests in trees. Stock dove (*Columba oenans*) - rare, but breeding bird. Turtle

Dove (*Streptopelia turtur*) - vagrant birds were usually seen in hilly areas. Cuckoo (*Cuculus canorus*) - seen daily, including foraging individuals in meadows and forested regions. Birds are very consistent in choice of habitat.

Strigiformes (Fig. 8), Piciformes (Figs. 9 and 10) and Apodiformes (Fig. 12)

Tawny owl *(Strix aluco) and T*engmalm´ s owl *(Ae*golius funereus) - regularly recorded the occurrence of both species, seen and heard in the forest. The Black woodpecker (*Dryocopus martius*) was mainly found in mixed beech and spruce forest habitats. The species was seen and heard daily, and when flying, it often crossed open hilly areas. Great gpotted woodpecker (*Dendrocops major*) and lesser spotted woodpecker (*Dendrocops minor*) - mainly preferred old beech forest while the three-toed woodpecker (*Picoides tridactylus*) was regularly seen in coniferous spruce forest. Swifts (*Apus apus*) - seen flying and feeding over open country.

Passeriformes

Alaudidae (Fig. 11). The skylark (*Alauda arvensis*) is one of the most notable birds of Veľký Brankov, breeding in the area‘s grasslands.

Hirundinidae (Fig. 12). Small numbers of swallows (*Hirundo rustica*) were seen most days. They were sometimes seen with house martins (*Delichon urbica*), flying over open country.

Motacillidae (Figs. 13-14). Tree pipit (*Anthus trivialis*) is a very abundant species on the main range of Veľký Brankov and Červená Magura. The species requires the presence of trees in breeding season, when it frequents open woodland. Meadow pipits (*Anthus pratensis*) - seen on the high meadows near the peak of Veľký Brankov. Rock pipit (*Anthus spinoletta*) - often seen in early spring, during migration. White wagtail (*Motacilla alba*) and grey wagtail (*Motacilla cinerea*) - usually observed near local springs and streams with fresh water.

Laniidae and Cinclidae (Fig. 1). Red-backed shrike (*Lanius collurio*) - a few individuals were reguralry seen over the mountain range during the breeding period. Dipper (*Cinclus cinclus*) was an occassional visitor of the mountain range.

Prunellidae (Fig. 15). Dunnock (*Prunella modularis*) - is one of the common birds breeding in the coniferous spruce forest. Four nests were found (Table 1).

Muscicapidae. Robin (*Erithacus rubecula*) - one of the most abundant species in the examined area (Fig. 16). Redstart (*Phoenicurus phoenicurus*) - males, in full song, were usually heard in old mixed beech and fir forest, adjacent to grassland areas (Fig. 17). Stonechat (*Saxicola torquatus*) - seen daily in the grasslands near Veľký Brankov hill (Fig. 18). Red-breasted flycatcher (*Ficedula parva*) - breeds in the natural beech-fir mixed forest, often heard singing when perched on branches of old, high trees (Fig. 29). Collared flycatcher (*Ficedula albicollis*) also seen breeding and singing in the old beech-fir forest while pied flycatcher (*Ficedula hypoleuca*) was recorded in early spring at the time of migration.

Turdidae (Figs. 19 - 22). The lightly wooded slopes, and meadows with grazing livestock are very suitable habitats for several species of thrushes - blackbird (*Turdus merula*), ring ouzel (*Turdus torquatus*), mistle thrush (*Turdus viscivorus*) and song thrush (*Turdus philomelos*). The nidobiology and postnatal development of the ring ouzel was the main aim of

Average number of laid eggs (*N=4)	Average number of hatched nestlings (*N=4)	Average number of fledglings (*N=3)	Number of measured eggs	Mean length +- SD (mm)	Mean width +- SD (mm)
4.75	3.5	3	10	20.07 +- 0.99	14.58 +- 0.40

Table 1. Breeding success rate of *Prunella modularis* (1985 - 1991 Brankov) *N - number of nests.

Average number of laid eggs (*N=5)	Average number of hatched nestlings (*N=4)	Average number of fledglings (*N=4)	Number of measured eggs	Mean length +- SD (mm)	Mean width +- SD (mm)
4	4	0.75	6	31.29 +- 1.30	21.25 +- 0.99

Table 2. Breeding success of *Turdus merula* (1985 - 1991 Brankov) *N - number of nests.

Average number of laid eggs (*N=11)	Average number of hatched nestlings (*N=7)	Average number of fledglings (*N=5)	Number of measured eggs	Mean length +- SD (mm)	Mean width +- SD (mm)
4.63	2	1	45	27.16 +- 0.96	20.56 +- 0.73

Table 3. Breeding success of *Turdus philomelos* (1985 - 1991 Brankov) *N - number of nests.

our research in this area. The results were, and will be, published in special studies dealing with this species. We estimate that approximately 40 males were singing in the area. Summarized records on the breeding rate of blackbird and song thrush are presented in Tables 2 and 3. Pine marten and red squirrel were the main predators of thrush nests. Fieldfare (*Turdus pilaris*) was rarely seen when crossing the mountain range

Sylviidae (Figs. 23-26). Sylvia warblers were recorded in tangled vegetation, scrub and low beech trees in the ecotone area between forest and grassland. A number of young birds of lesser whitethroat (*Sylvia curruca*) and blackcap (*Sylvia atricapilla*) species were also seen (see also Table 4). Leaf warblers - chifchaff (*Phylloscopus collybita*), willow warbler (*Ph. trochillus*) and wood warbler (*Ph. sibilatrix*) were very plentiful species. The wood warbler breeds in mixed beech-spruce forest while chifchaff and wilow warbler were seen and heard in similar woodland habitats with scattered trees

Regulidae (Fig. 27). Goldcrest (Regulus regulus) is a very common species living in coniferous spruce forest. Firecrest (*Regulus ignicapilla*) preferred old fir conifers mixed with beech.

Troglodytidae (Fig. 28). Wren (*Troglodytes troglodytes*) - seen and heard daily.

Paridae (Figs. 30-34). Tit abundance and density in a particular habitat is believed to be regulated by a vast combination of factors interacting with one another. This becomes apparent when one examines the breeding bird community of a particular habitat and discovers that year by year it is a dynamic system. Any seasonal or yearly variation of that habitat may result in changes to the suitability of the habitat for a tit species´ niche requirements. In our case, the great tit (*Parus major*) was drastically affected by seasonal fluctuations between different years. In nest boxes, the number of breeding birds greatly fluctuated between high numbers to zero, while other tit species like the marsh tit (*Poecile palustris*) and willow tit (*Poecile montanus*) remained relatively stable. Breeding success of tit pairs nesting in the nest boxes is presented in Tables 5, 6, and 7.

Aeghitalidae (Fig. 35). Long-tailed tit (*Aegithalos caudatus*) - frequently observed in the lower broadleafed and mixed woods and scrub.

Sittidae (Fig. 36). Nuthatch (*Sitta europaea*) - common, seen daily but mainly in old mixed fir-beech forest. A female, breeding in a nestbox, laid eight eggs, from which seven young hatched and fledged.

Certhiidae (Fig. 37). Treecreeper (*Certhia familiaris*). The species is common in spruce, fir and mixed wodland, resident, often with tit parties in winter.

Corvidae (Figs. 38-39). Nutcracker (*Nucifraga caryo-*

Average number of laid eggs	**Average number of hatched nestlings**	**Average number of fledglings**	**Number of measured eggs**	**Mean length +- SD (mm)**	**Mean width +- SD (mm)**
5	4	4	5	19.94 +- 0.51	14.41 +- 0.14

Table 4. Breeding success and egg size of a pair of *Sylvia atricapilla*.

Average number of laid eggs (*N=5)	**Average number of hatched nestlings (*N=5)**	**Average number of fledglings (*N=5)**	**Number of measured eggs**	**Mean length +- SD (mm)**	**Mean width +- SD (mm)**
12	10.4	8.8	46	17.42 +- 1.64	13.75 +- 1.38

Table 5. Breeding success of *Parus major* (1985 - 1991 Brankov) *N - number of nests.

Average number of laid eggs (*N=5)	**Average number of hatched nestlings (*N=5)**	**Average number of fledglings (*N=5)**	**Number of measured eggs**	**Mean length +- SD (mm)**	**Mean width +- SD (mm)**
8.4	7.8	7.4	10	16.36 +- 0.27	11.85 +- 0.14

Table 6. Breeding success of *Periparus ater* (1985 - 1991 Brankov) *N - number of nests.

Average number of laid eggs (*N=2)	**Average number of hatched nestlings (*N=2)**	**Average number of fledglings (*N=2)**	**Number of measured eggs**	**Mean length +- SD (mm)**	**Mean width +- SD (mm)**
8	4	4	8	15.75 +- 0.14	12.28 +- 0.18

Table 7. Breeding success of *Poecile montanus* (1985 - 1991 Brankov) *N - number of nests.

catactes) - seen daily, calling from the tops of Norway spruce on the lower slopes of Veľký Brankov and Červená Magura. Young birds were also recorded. Jay (*Garrulus glandarius*) - seen usually individually or in small parties in the forested areas with numerous scattered trees. Raven (*Corvus corax*) - seen often, particularly in early spring when it is common for them to flock, but during breeding season, commonly observed individually, in pairs or in family parties. Veľký Brankov is a hunting area for this species; birds nested on rock ledges in the neighboring valley. Hooded crow *(Corvus corone cornix*) - vagrants seen in open country.

Fringillidae (Figs. 40-44). Chaffinch (*Fringilla coelebs*) - is the most common passerine species, and is observed in a wide variatey of habitats, often in woods and areas with scattered trees. Breeding success of one pair is presented in Table 8.

Linnet (*Carduelis cannabina*), goldfinch (*Carduelis carduelis*), and greenfinch (*Chloris chloris*) were frequently found in habitats with scattered trees while siskin (*Spinus spinus*) was recorded in spruce and mixed forest. Hawfinch (*Coccothraustes coccothraustes*) was seen in old fir/beech mixed forest. Bullfinch (*Pyrhulla pyrhulla*) and common crossbill (*Loxia curvirostra*) were widely distributed, and most common in mixed and spruce forest, respectively.

Emberizidae. Yellowhammer (*Emberiza citrinella*) - usually found and heard in sunny grassland habitats with scattered trees. Good local population was recorded near Červená Magura.

In summary, each of the four distict habitats in the Brankov area - grassland, woodland with scattered trees (ecotone), spruce, old mixed fir-beech forest - has its own characteristic birds. Climate and topography, through their influence on vegetation, are the prime factors in the distribution and kinds of birds observed in these habitats. Yearly, variations in breeding conditions surrounding some of the higher Low Tatra and Great Fatra mountains are likely barriers to movement of some species.

Acknowledgements

We are grateful to all our friends for accompanying the first author in the field and for their assistance and help in bird monitoring.

Average number of laid eggs	Average number of hatched nestlings	Average number of fledglings	Number of measured eggs	Mean length +- SD (mm)	Mean width +- SD (mm)
5	5	5	5	19.6 +- 0.66	15.2 +- 0.27

Table 8. Breeding success and egg dimensions of a pair of *Fringilla coelebs*.

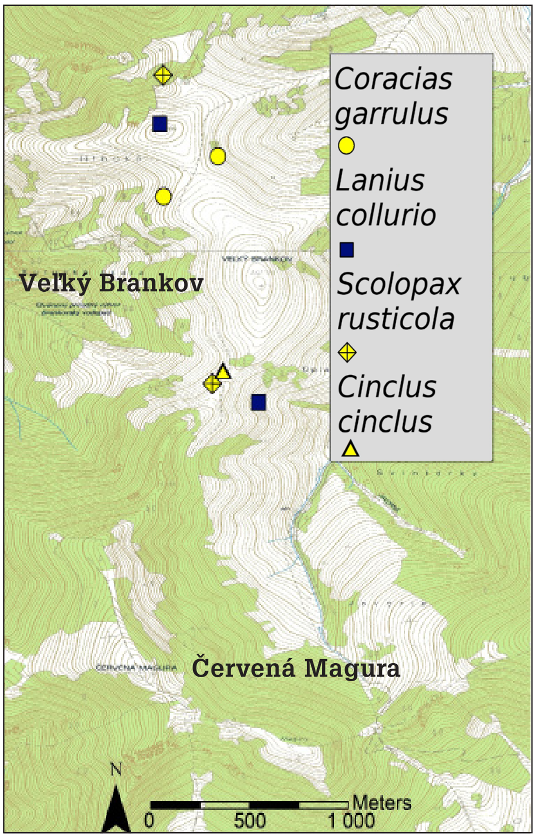

Fig. 1. Observations of rare species.

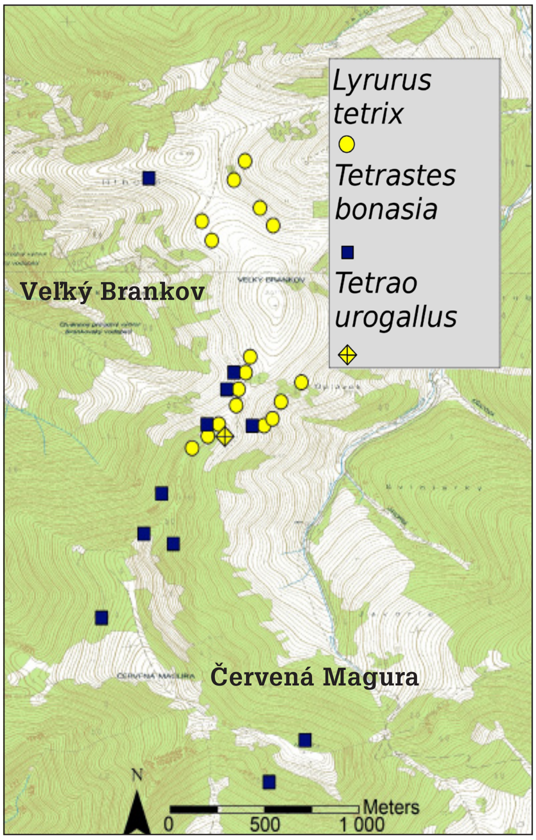

Fig. 2. Frequent occurrence of *Lyrurus tetrix, Tetrastes bonasia* and observations of *Tetrao urogallus.*

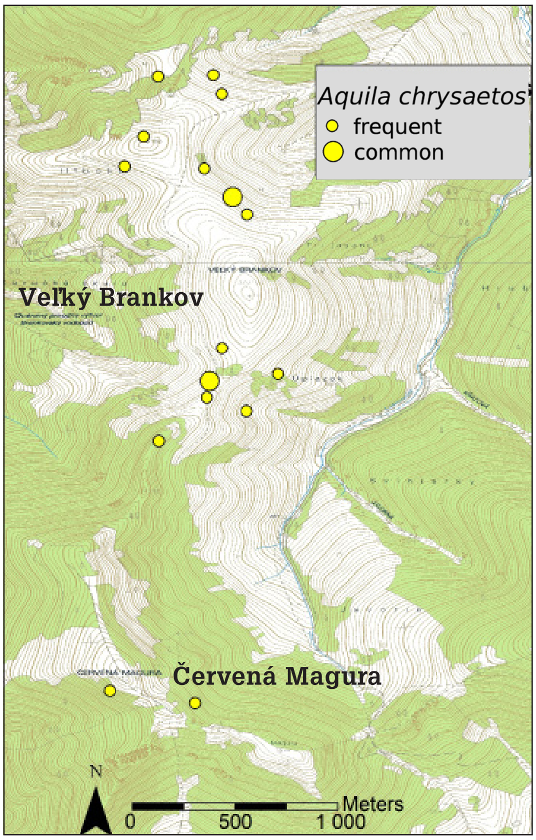

Fig. 3. Occurrence of *Aquila chrysaetos*.

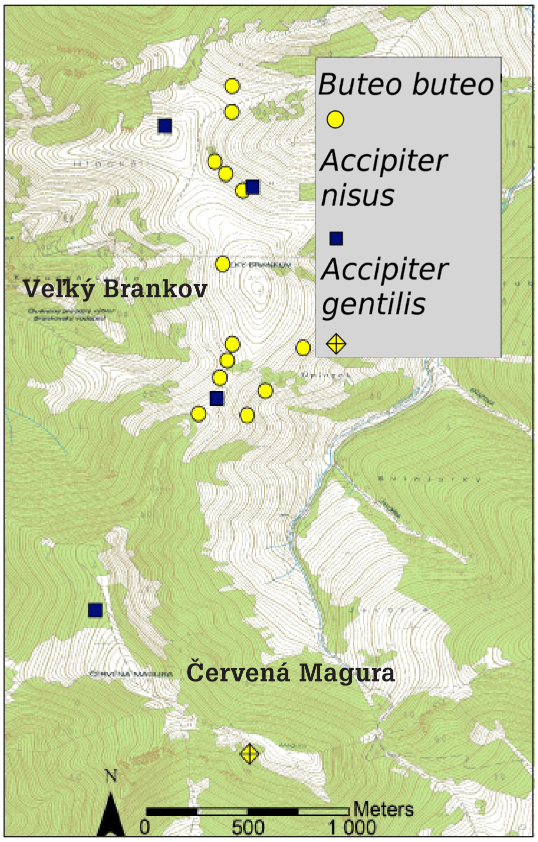

Fig. 4. Frequent occurrence of *Buteo buteo*, *Accipiter nisus* and obseravtions of *Accipiter gentilis*.

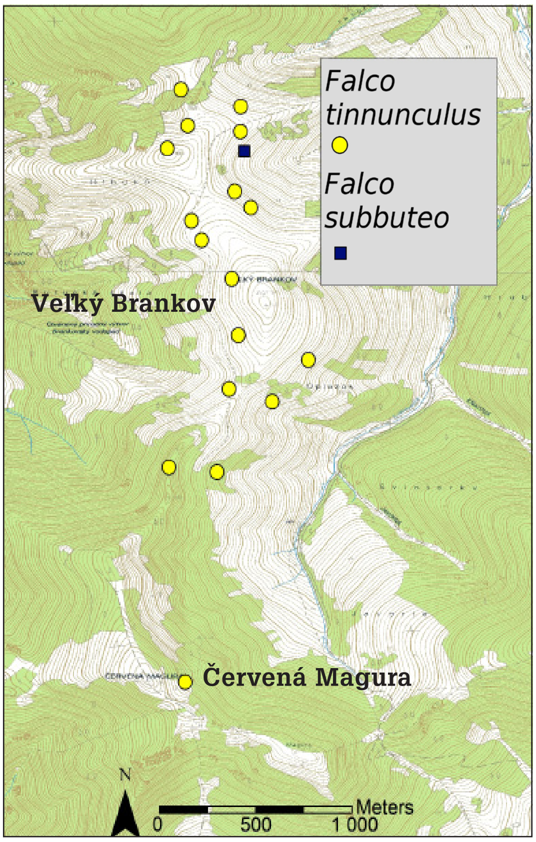

Fig. 5. Frequent occurrence of *Falco tinnunculus* and observations of *Falco subbuteo*.

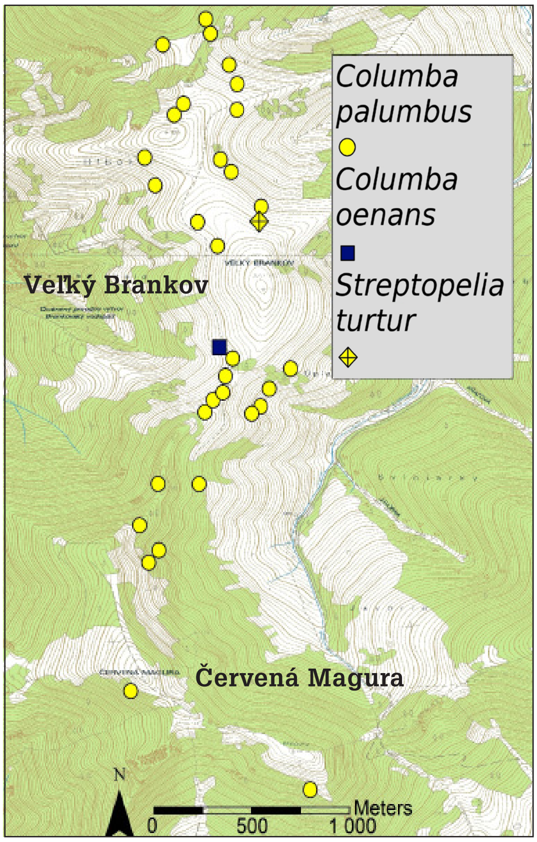

Fig. 6. Frequent occurrence of *Columba palumbus* and observations of *Columba oenans* and *Streptopelia turtur*.

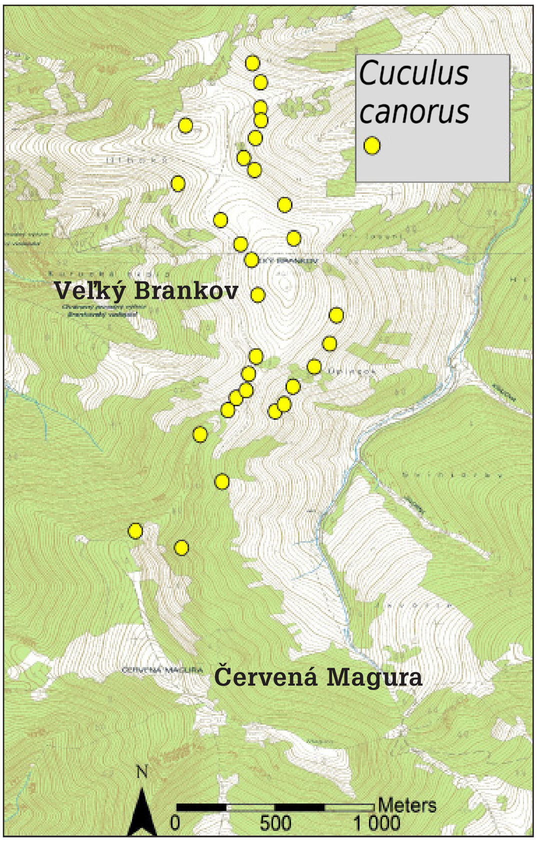

Fig. 7. Frequent occurrence of *Cuculus canorus*.

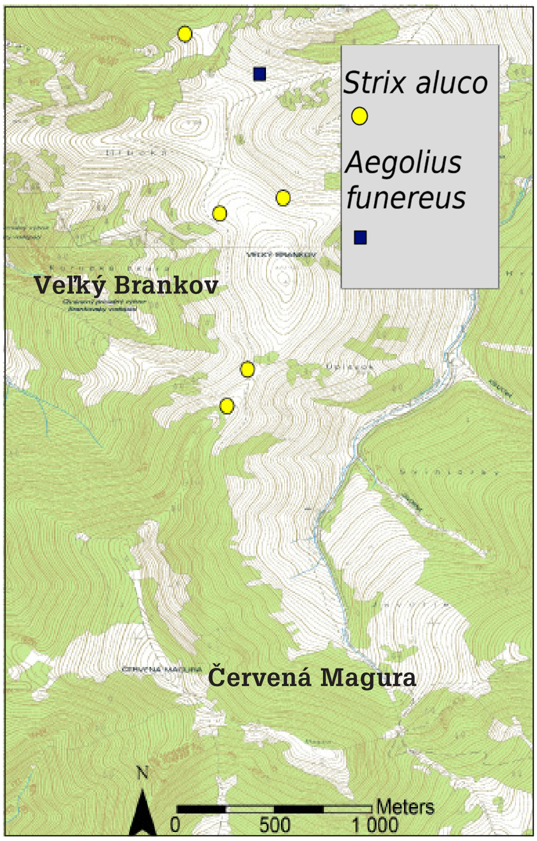

Fig. 8. Observations of *Strix aluco* and *Aegolius funereus*.

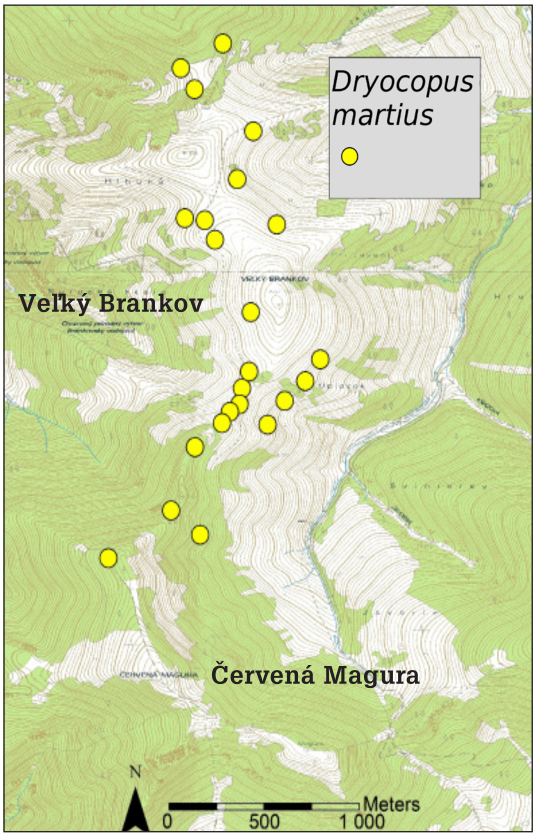

Fig. 9. Frequent occurrence of *Dryocopus martius*.

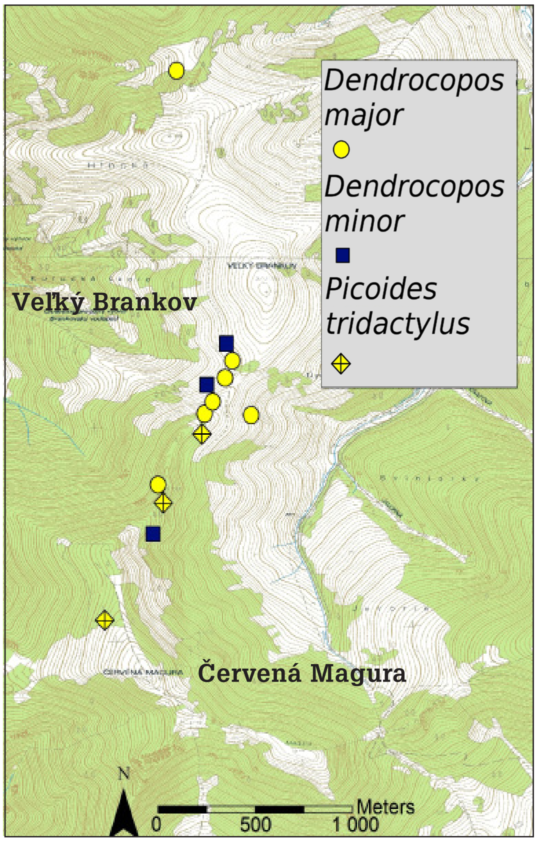

Fig. 10. Frequent occurrence of *Dendrocopos major* and observations of *Dendrocopos minor* and *Picoides tridactylus*.

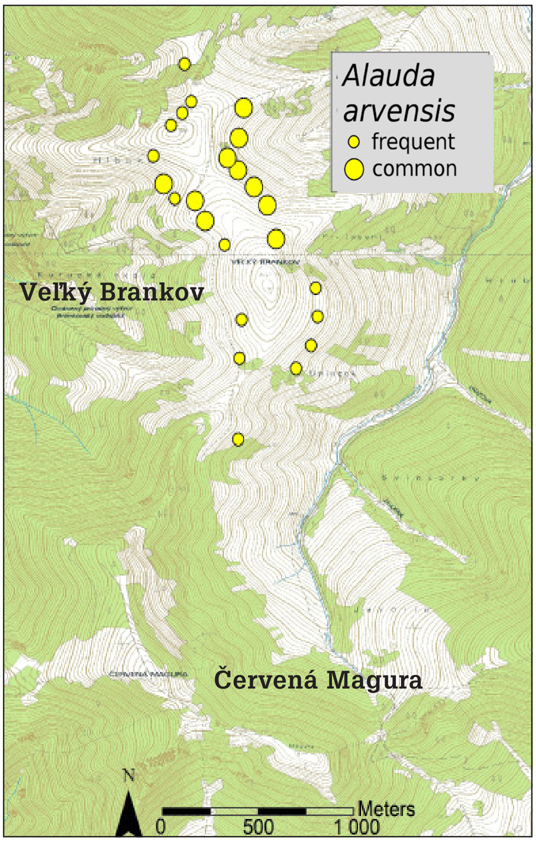

Fig. 11. Occurrence of *Alauda arvensis*.

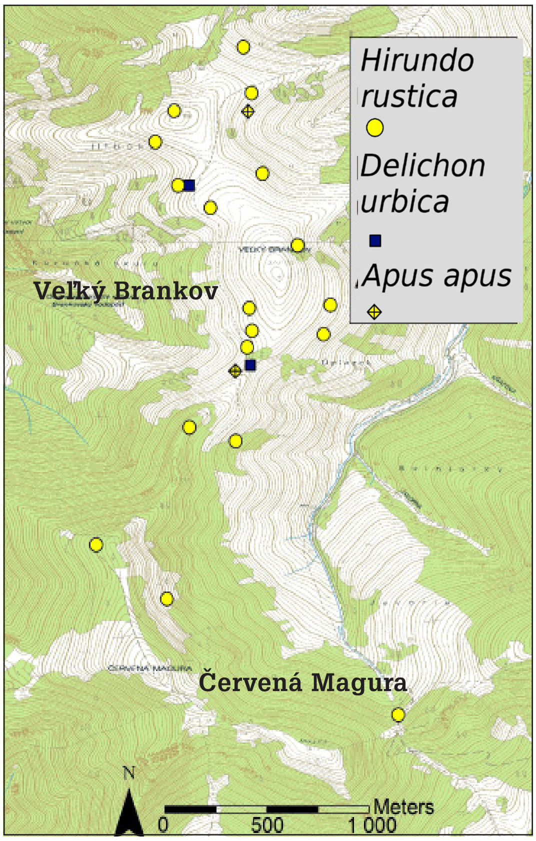

Fig. 12. Frequent occurrence of *Hirundo rustica*, *Delichon urbica* and *Apus apus*.

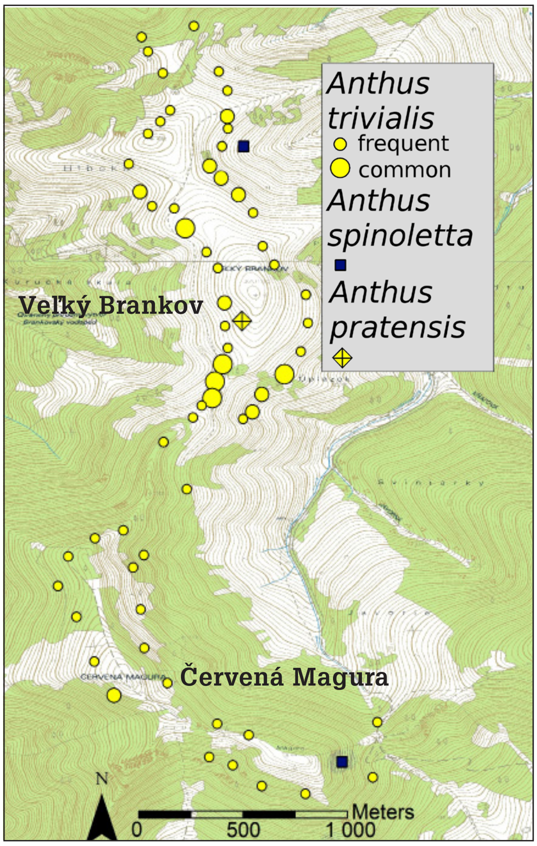

Fig. 13. Occurrence of *Anthus trivialis* and observations of *Anthus spinoletta* and *Anthus pratensis*.

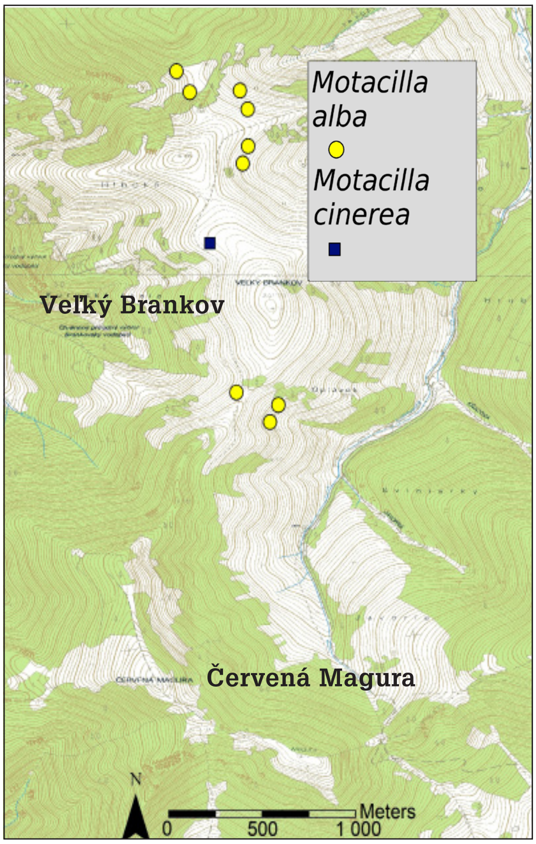

Fig. 14. Frequent ccurrence of *Motacilla alba* and observations of *Motacilla cinerea*.

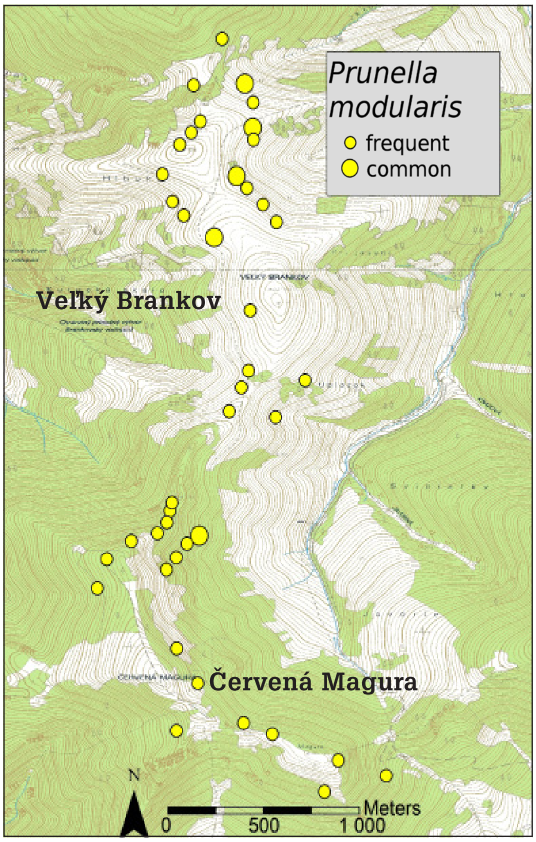

Fig. 15. Occurrence of *Prunella modularis.*

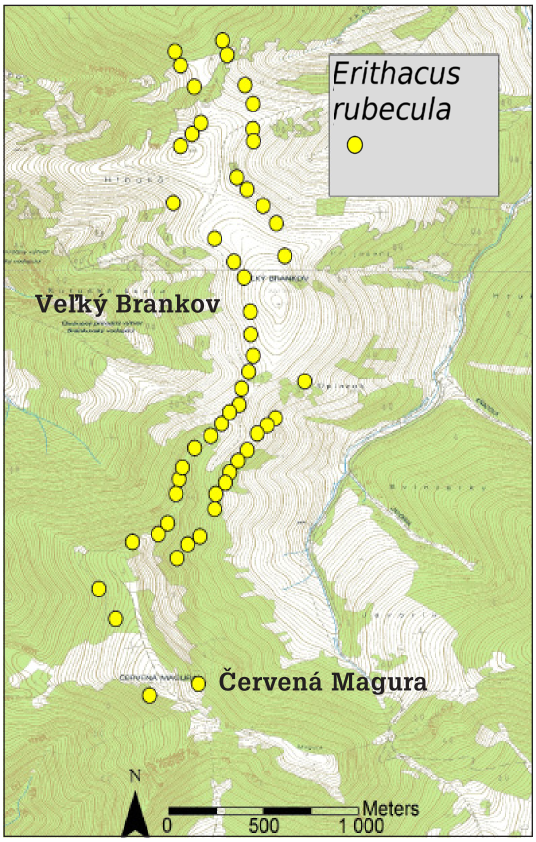

Fig. 16. Frequent occurrence of *Erithacus rubecula.*

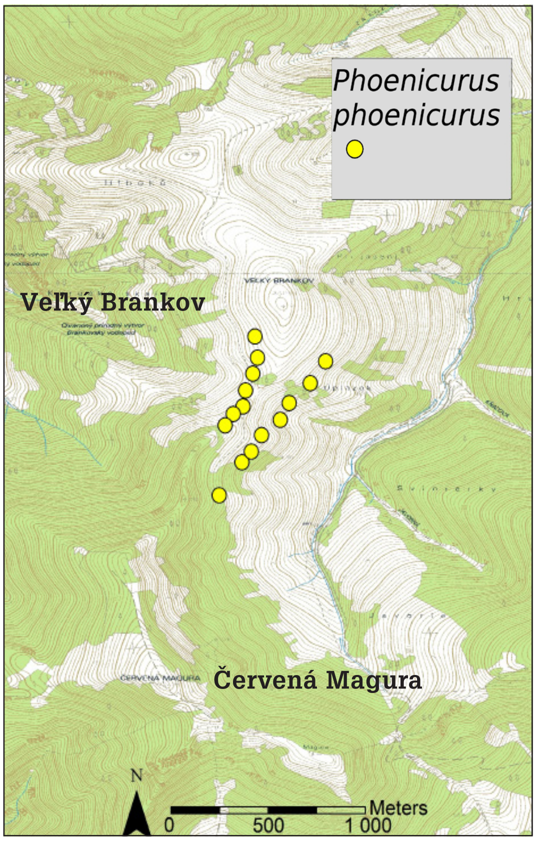

Fig. 17. Frequent occurrence of *Phoenicurus phoenicurus.*

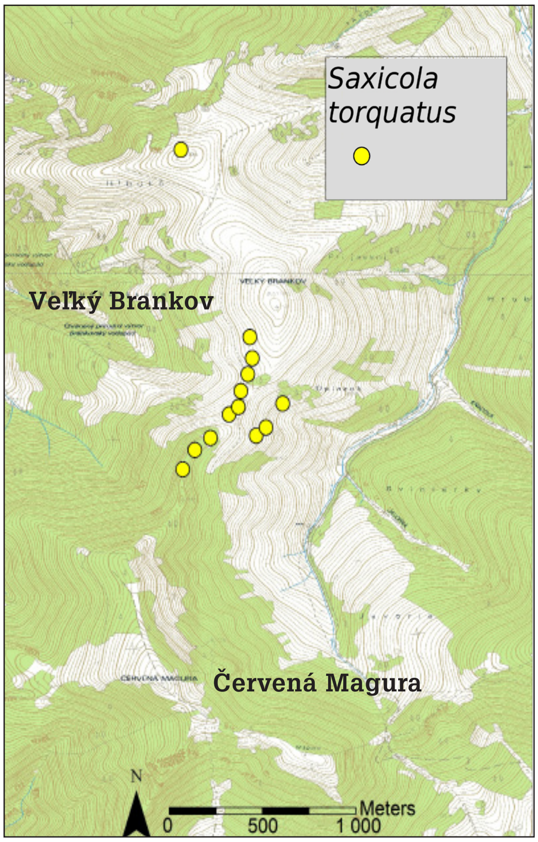

Fig. 18. Frequent occurrence of *Saxicola torquatus.*

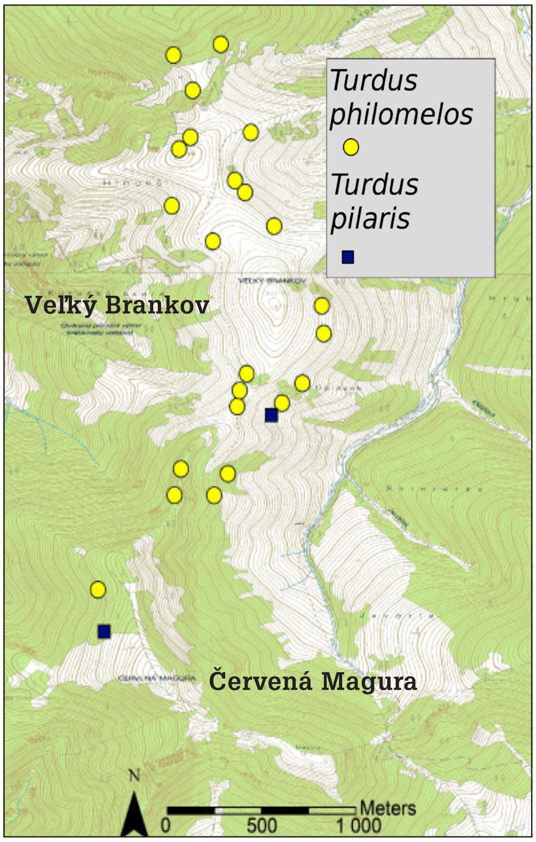

Fig. 19. Frequent occurrence of *Turdus philomelos* and *Turdus pilaris*.

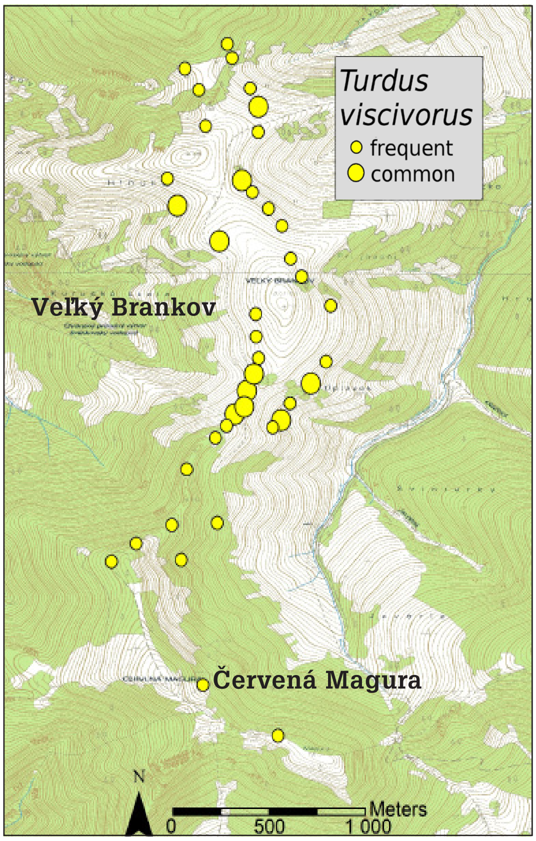

Fig. 20. Occurrence of *Turdus viscivorus*.

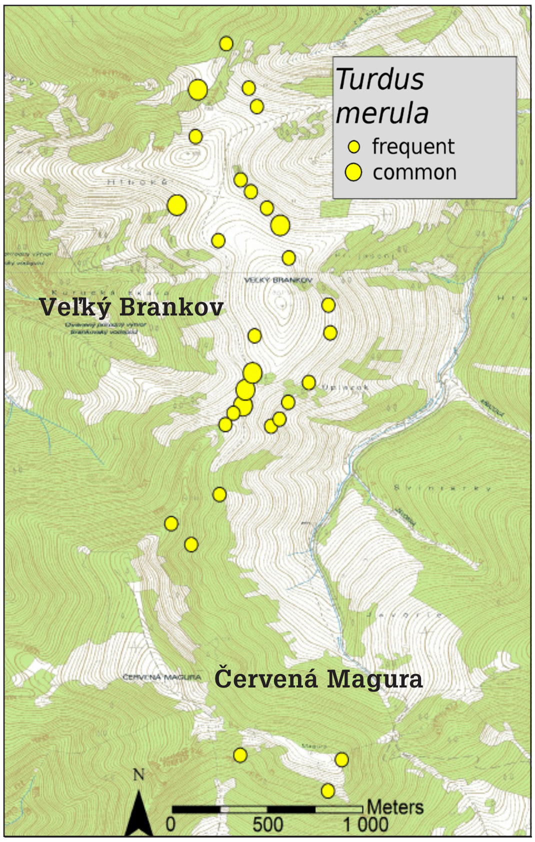

Fig. 21. Occurrence of *Turdus merula*.

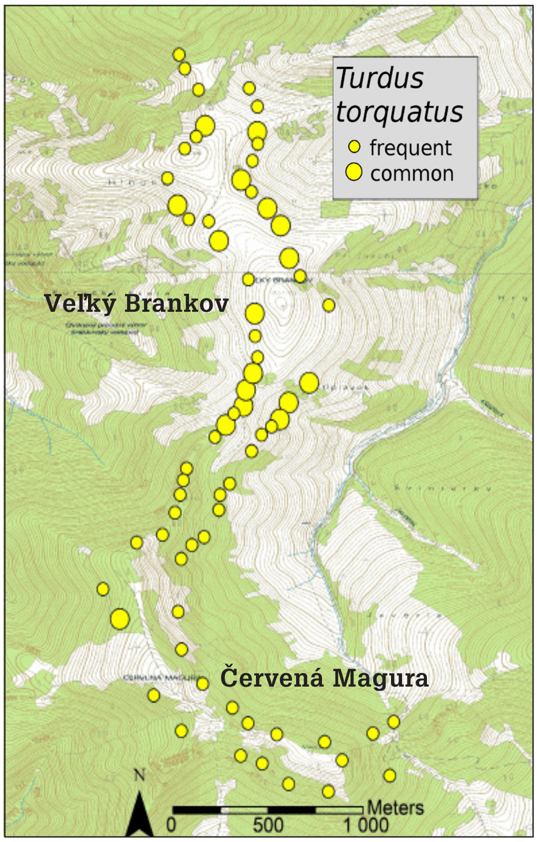

Fig. 22. Occurrence of *Turdus torquatus*.

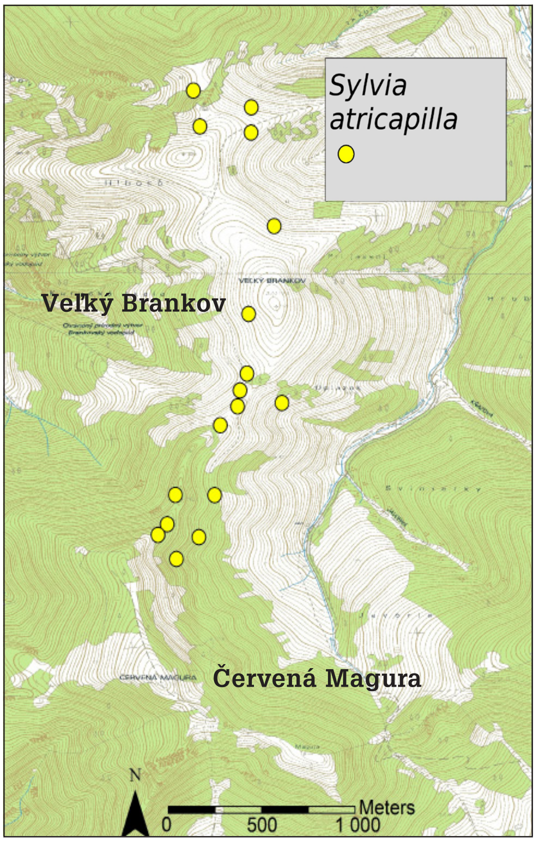

Fig. 23. Frequent occurrence of *Sylvia atricapilla*.

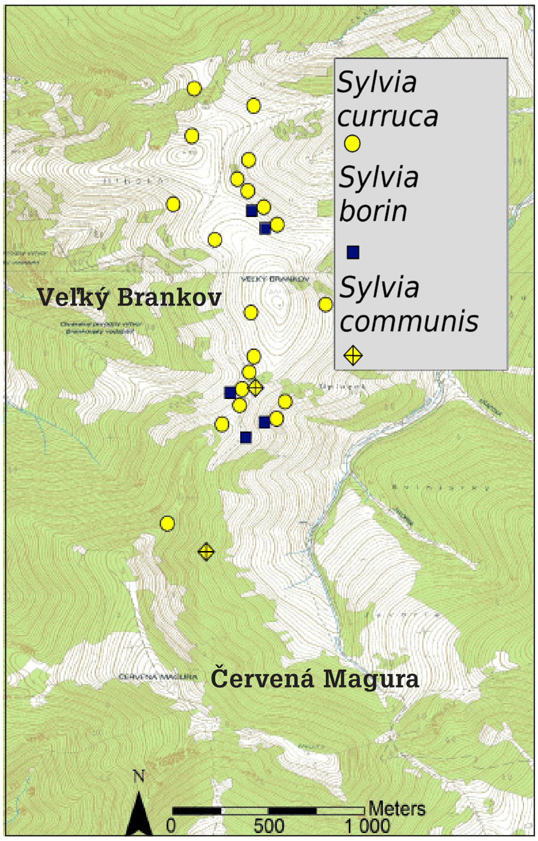

Fig. 24. Frequent occurrence of *Sylvia curruca*, *Sylvia borin* and *Sylvia communis*.

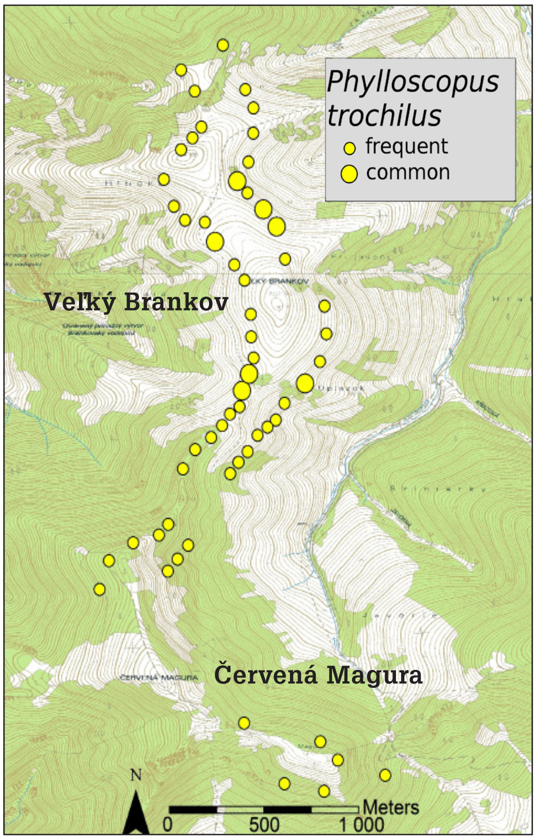

Fig. 25. Occurrence of *Phylloscopus trochilus*.

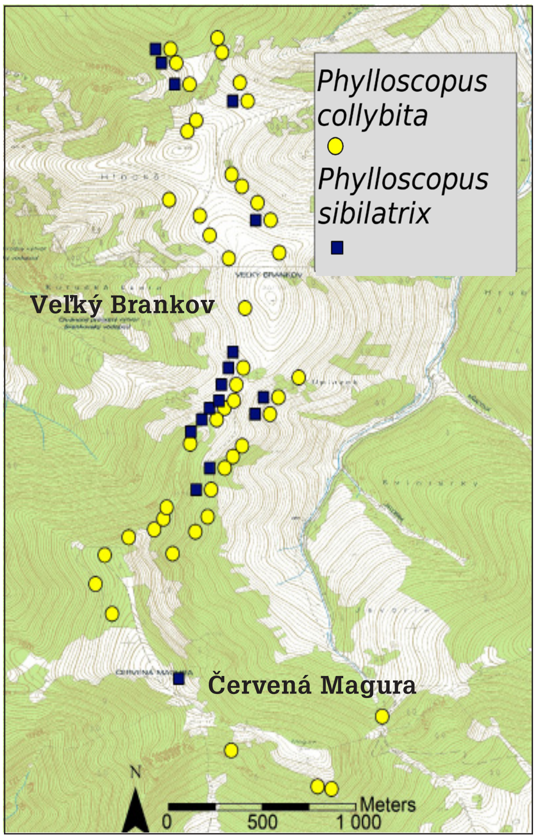

Fig. 26. Frequent occurrence of *Phylloscopus collybita* and *Phylloscopus sibilatrix*.

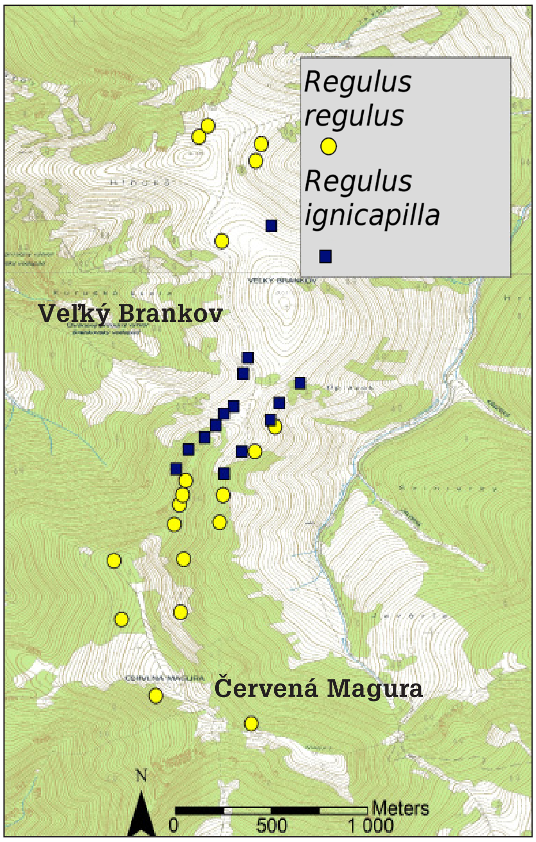

Fig. 27. Frequent occurrence of *Regulus regulus* and *Regulus ignicapilla*.

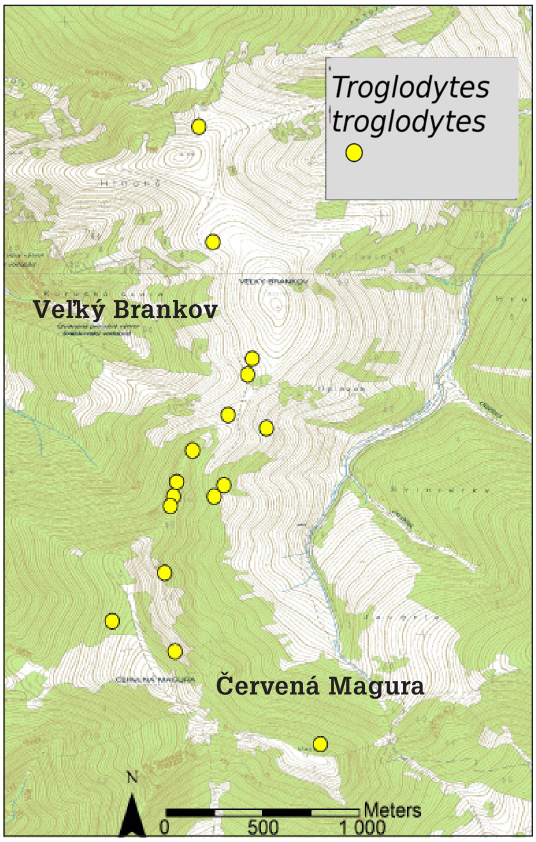

Fig. 28. Frequent occurrence of *Troglodytes troglodytes*.

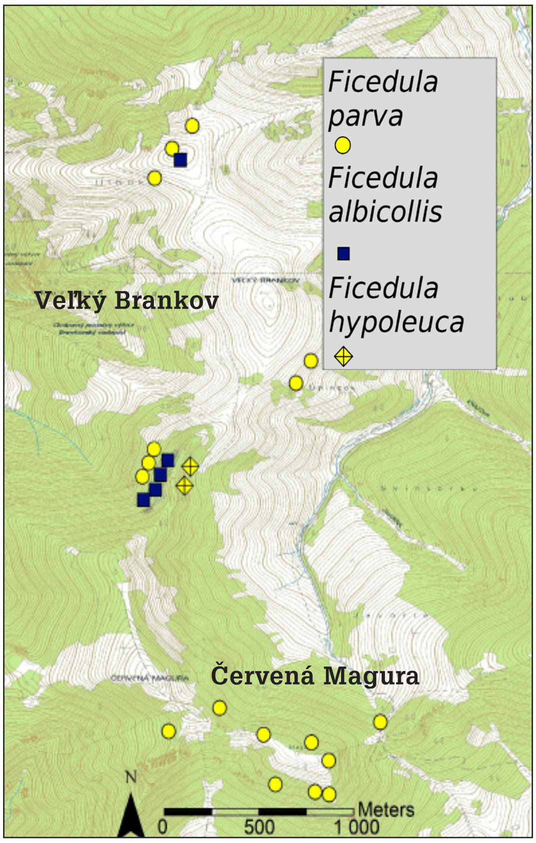

Fig. 29. Frequent occurrence of *Ficedula parva*, *Ficedula albicollis* and *Ficedula hypoleuca*.

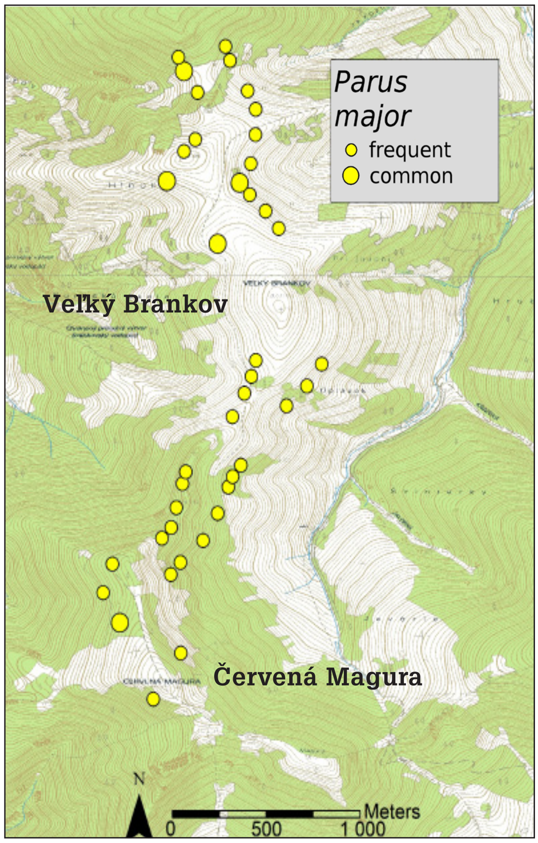

Fig. 30. Occurrence of *Parus major*.

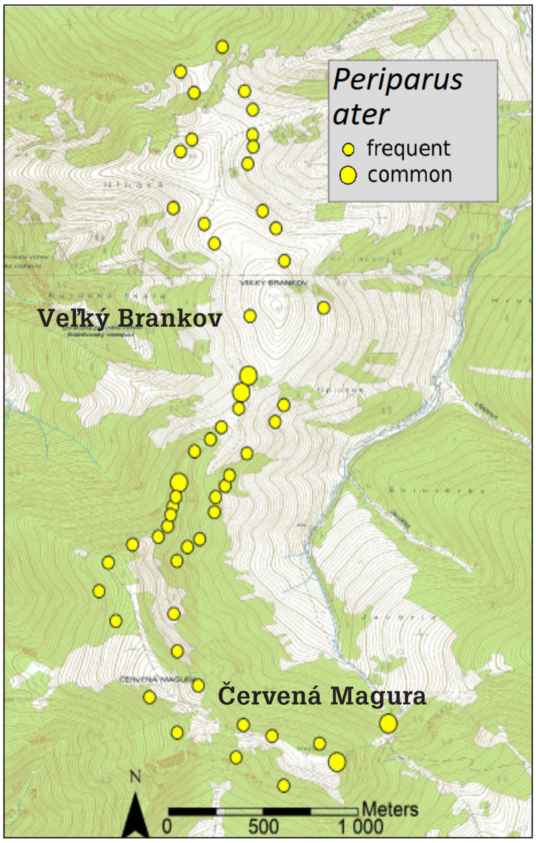

Fig. 31. Occurrence of *Periparus ater*.

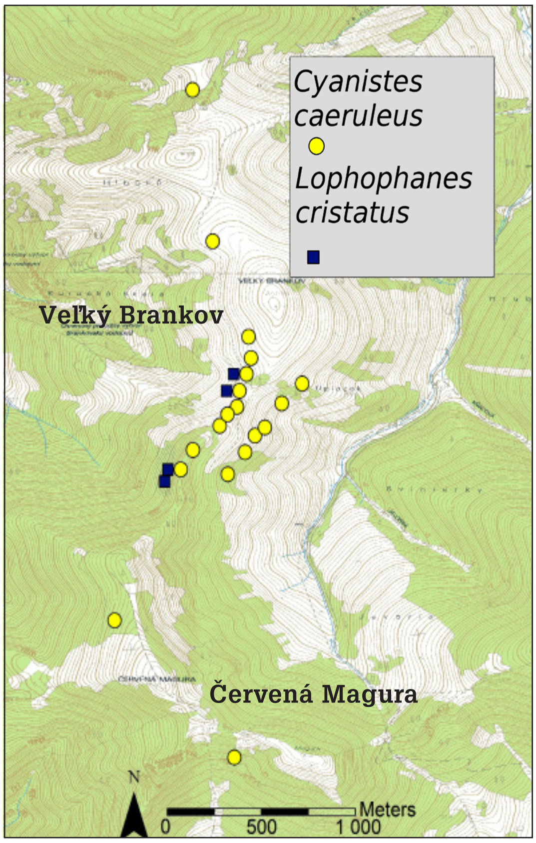

Fig. 32. Frequent occurrence of *Cyanistes caeruleus* and *Lophophanes cristatus*.

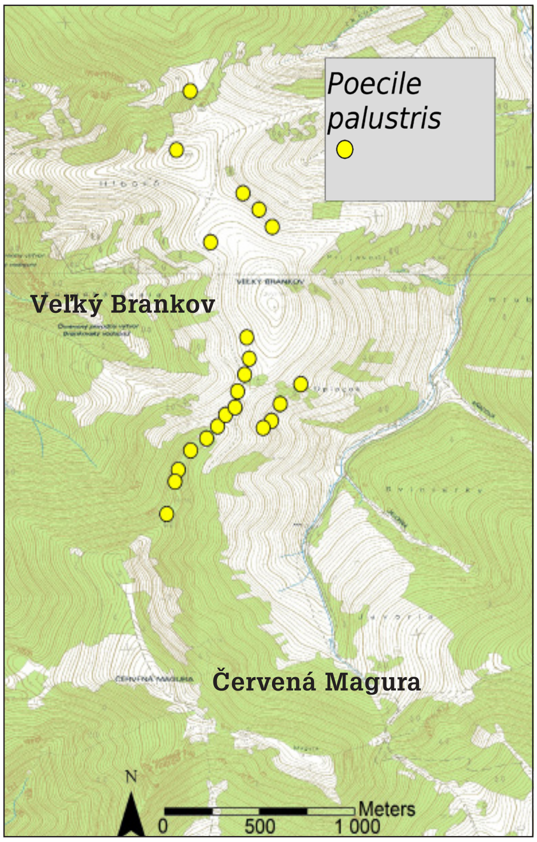

Fig. 33. Frequent occurrence of *Poecile palustris*.

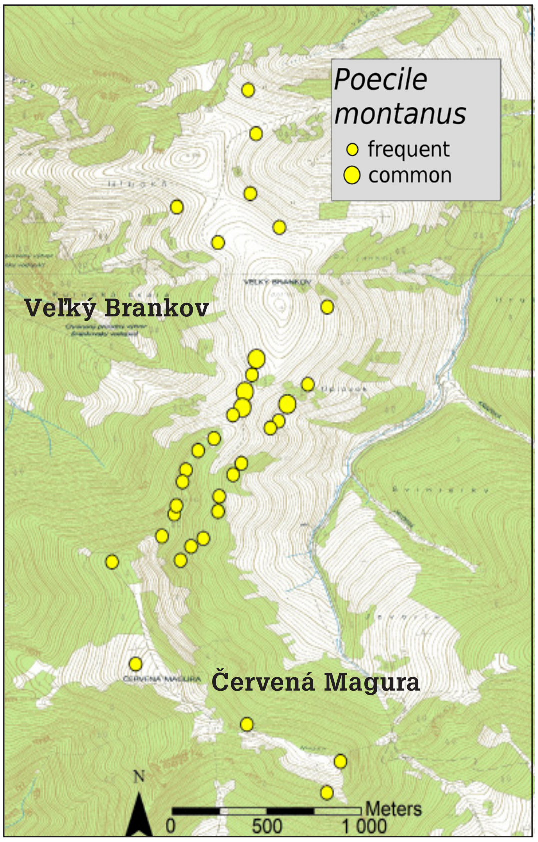

Fig. 34. Frequent occurrence of *Poecile montanus*.

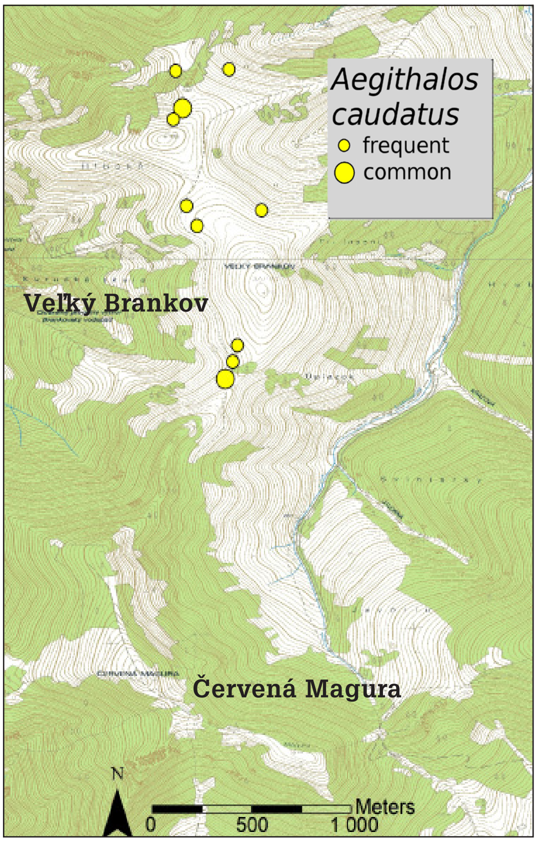

Fig. 35. Occurrence of *Aegithalos caudatus*.

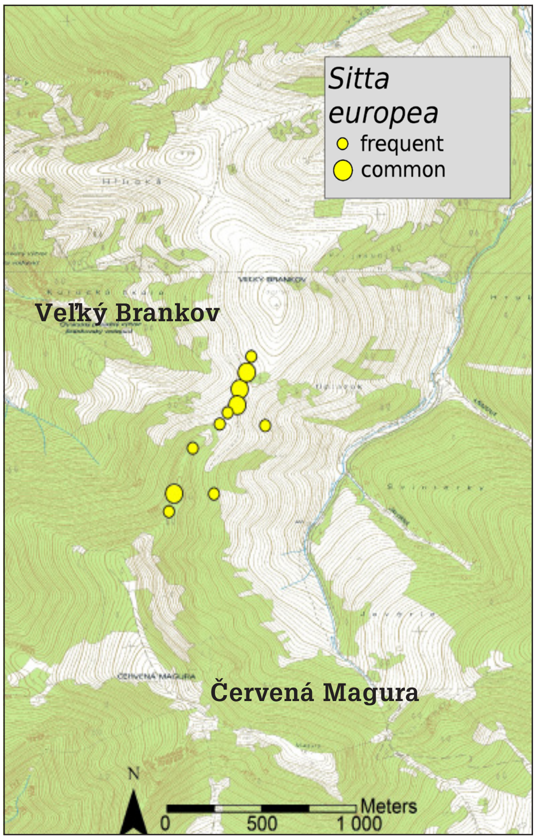

Fig. 36. Occurrence of *Sitta europea*.

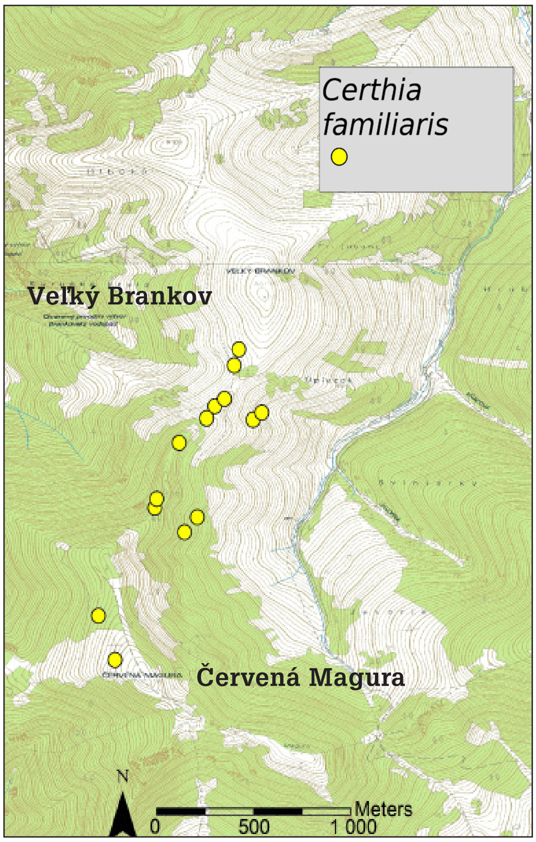

Fig. 37. Frequent occurrence of *Certhia familiaris*.

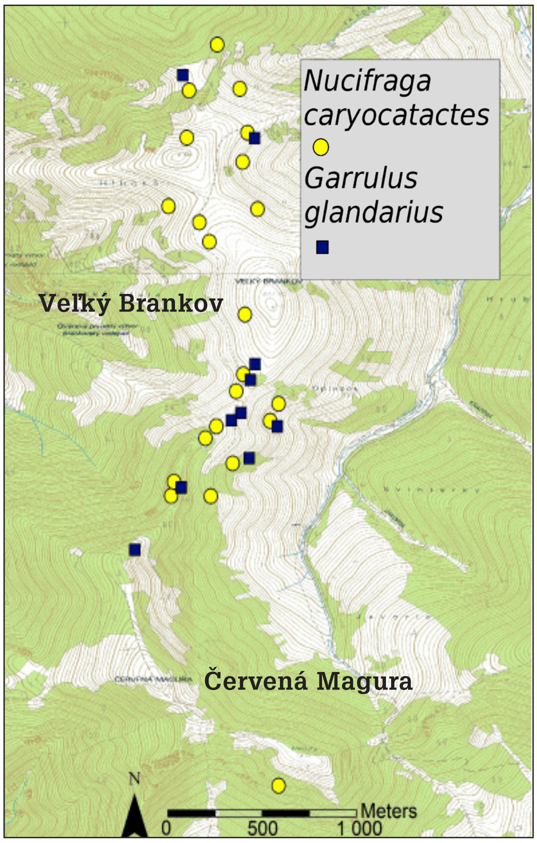

Fig. 38. Frequent occurrence of *Nucifraga caryocatactes* and *Garrulus glandarius*.

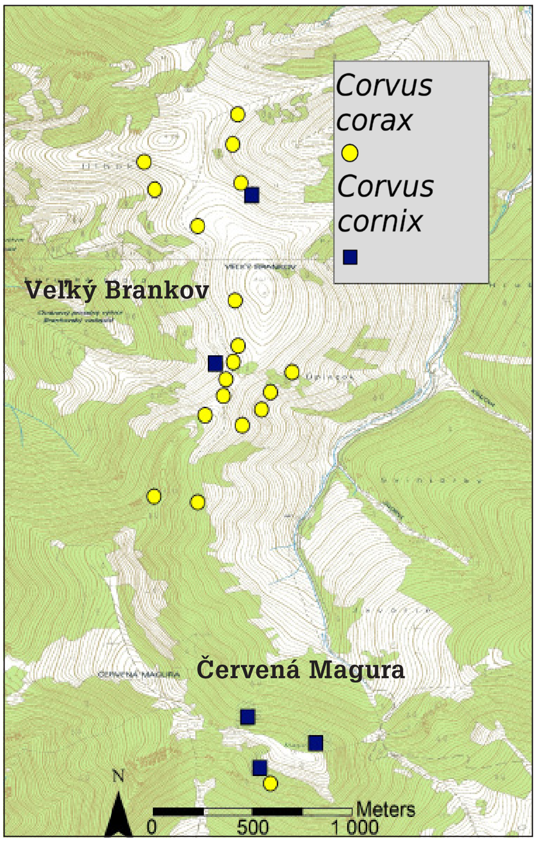

Fig. 39. Frequent occurrence of *Corvus corax* and *Corvus cornix*.

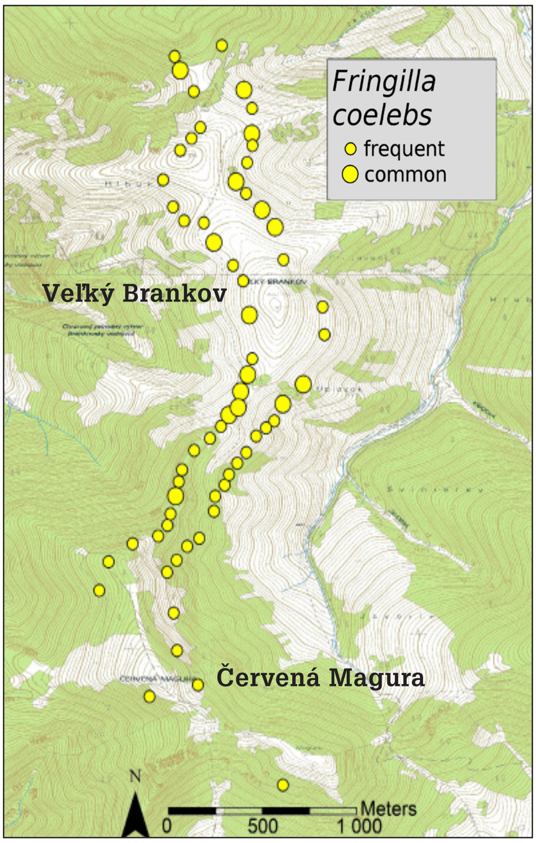

Fig. 40. Occurrence of *Fringilla coelebs*.

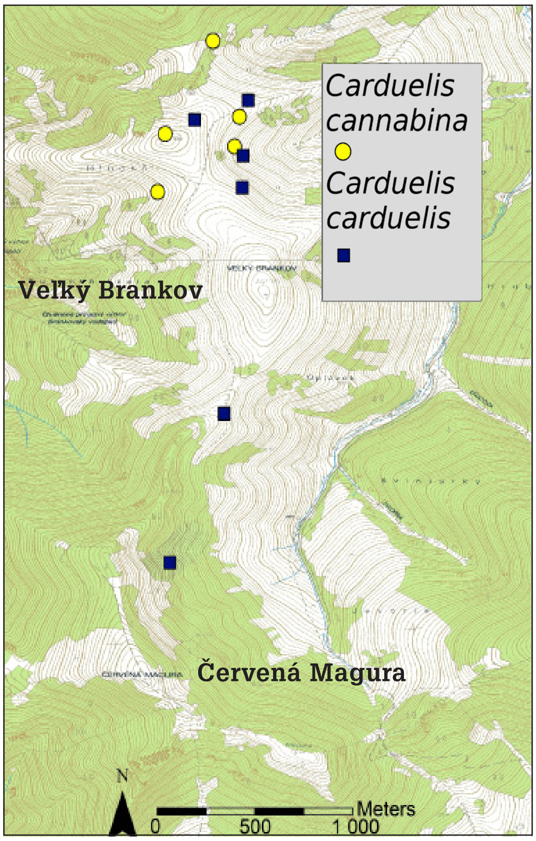

Fig. 41. Frequent occurrence of *Carduelis cannabina* and *Carduelis carduelis*.

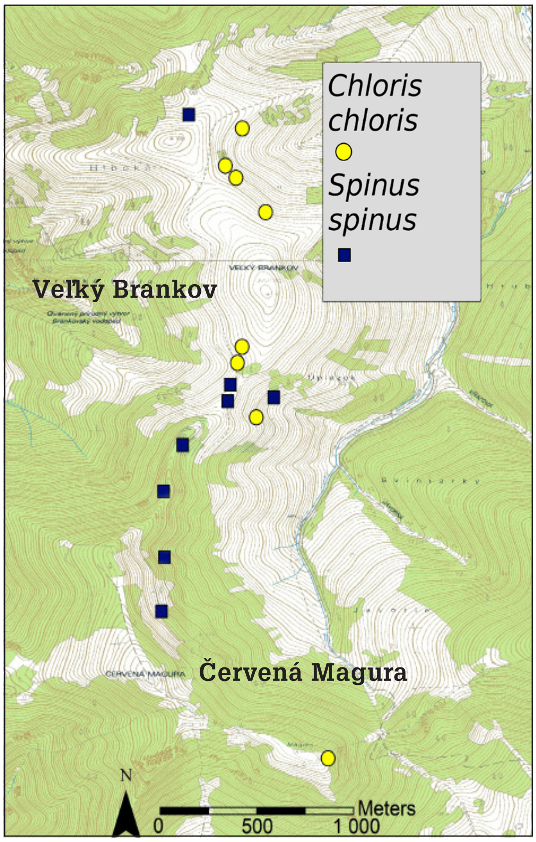

Fig. 42. Frequent occurrence of *Chloris chloris* and *Spinus spinus*.

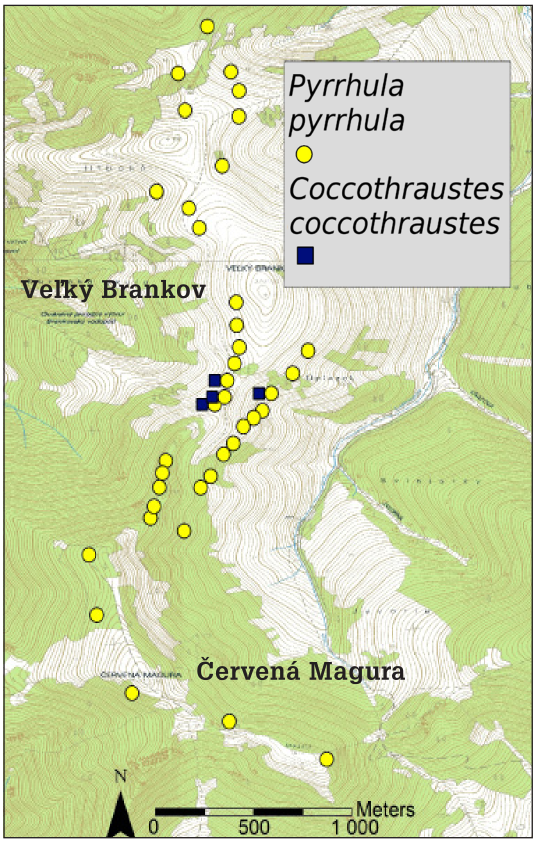

Fig. 43. Frequent occurrence of *Pyrrhula pyrrhula* and *Coccothraustes coccothraustes*.

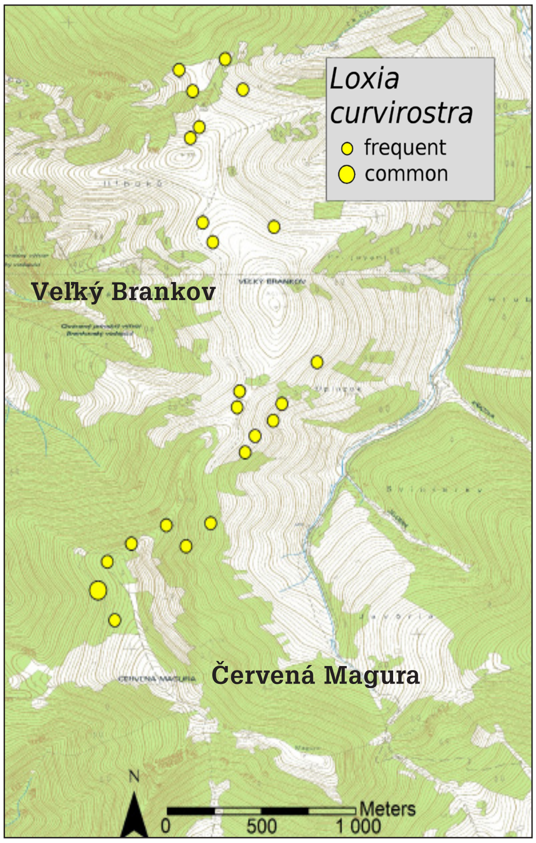

Fig. 44. Occurrence of *Loxia curvirostra*.

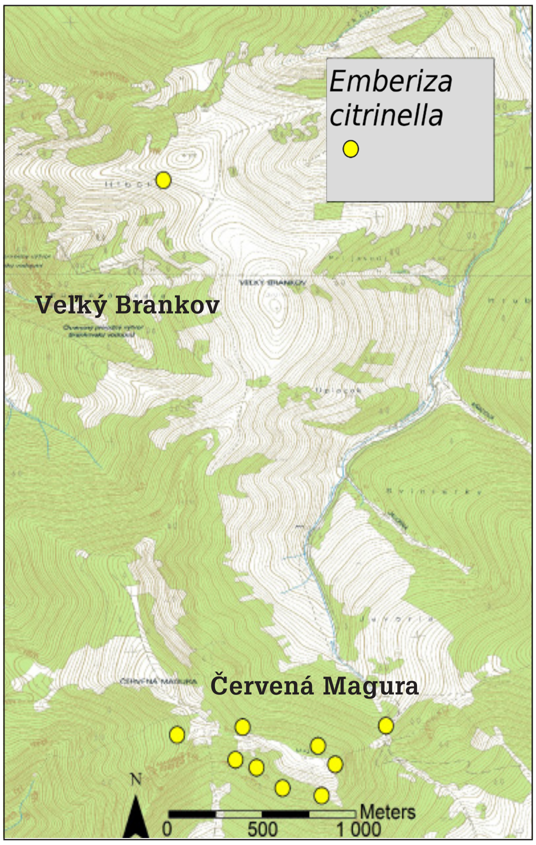

Fig. 45. Frequent occurrence of *Emberiza citrinella*.

References:

Johnston, D.W. and Odum, E.P. 1956: Breeding bird ppulations in relation to plant succession in the Piedmont of Georgia. *Ecology*, **37**: 50-62.

Karr, J.R. 1971: Structure of avian communities in selected Panama and Illinois habitats. *Ecol. Monogr.*, **41**: 207-233.

MacArthur, R.H. 1965: Patterns of species diversity. *Biol. Rev.*, **40**: 510-533.

Moskát, C., Hrasko, G. and Walitzky, Z. 1988: Species composition and the structure of avian communities in the Pila Mountains, North Hungary. In: *Ornithological researches in the Pilis Biosphere Reserve* (ed. J. Torok), pp. 12-20. Hungarian Ornithological Society, Budapest.

Payne, R.B. 1982: Species limits in the Indigobirds (Ploceidae, Vidua) of west Africa: Mouth mimicry, song mimicry, and description of new species. *Misc. Publ. Museum of Zoology, Univ. of Michigan*, **162**: 1-96.

Received 18 August 2019; accepted 3 October 2019.