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Trace elements in soil and mosses (*Dicranoweisia crispula* (Hredw.) Milde) by the road to Stelvio pass, Northern Italy Apls: A case of small scale pollution biomonitoring

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Abstract. The moss species *Dicranoweisia crispula* for bioindication were collected together with soil along road to the second highest paved mountain pass (Passo dello Stelvio) in the Alps. The trace elements such as P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Sn, Sb, Ba and Pb were determined using the hand-held XRF spectrometer DELTA CLASSIC (USA). From principal component analysis we determined four main phenomena, which were then evaluated in the context of localisation by the road. Several phenomena were described which could be ubiquitous in mountain regions, such as transboundary emission and traffic pollution related to touristic pressure.

Key words: bioindication, mosses, heavy metals, X-ray spectrometry, Italy, Stelvio paas

Introduction

The use of mosses as bioindicators of environmental pollution gained broadly attention, as a successful model for studying spatial pollution of environment from various sources, such as from minerals in geological parent material and inputs from wide range of possible antropogenic sources. The whole idea use bryophytes as bioindicators arose in seventies (Clymo 1963; Rühling and Tyler 1970) and immediately has spread across all Europe and the USA (Gordon *et al.* 1971; Briggs 1972; Grözinger 1974; Ratcliffe 1975; Groet 1976; Pilegaard *et al.* 1979; Gydesen and Rasmussen 1981; Sumerling 1984; Zoltai 1988; Burton 1990; Šoltés 1992; Zechmeister 1994). This simple model is based on the fact that, bryophytes are incapable of avoiding heavy metal uptake from deposition due great exchange capacity, absence of a cuticle and a simple organisation of the tissue (Tyler 1990; Sabovljević *et al.* 2005). From nutritional point of view, the bryophytes are independent of the soil (Ratcliffe 1975; Tyler 1990; Burton 1990). The cation exchange capacity is related to the concentration of peptic substances in moss tissue (Šoltés and Gregušková 2013). Mosses do not have real roots, epidermis or continuous cuticle layer, and they absorb water and dissolved elements directly across their surface (Salemaa *et al.* 2004). Therefore, moss species can be used as biological samplers for dry, wet, and occult deposition over a long period of time (Meyer *et al.* 2015; Gonzales and Pokrovsky 2014) and furthermore, they are rather resistant to toxic elements such as heavy metals (Berg and Steinnes 1997; Basile *et al.* 2013). Currently, surveys of the atmospheric deposition of trace element through mosses, using the moss biomonitoring technique are regularly performed in several European countries every 5 years since 1990 (Harmens *et al.* 2015; Schröder *et al.* 2016) And the most important guideline for the application of the moss technique is "Monitoring of atmospheric deposition of heavy metals, nitrogen and POPs in Europe using bryophytes" published by the UNECE ICP Vegetation 2014 (ICP Vegetation 2014; Frontasyeva *et al.* 2014). During the European moss survey 2010, moss was sampled at 4.499 sites in 25 countries and 14 elements were determined (Schröder *et al.* 2016). The results revealed that in general, mosses from countries in Northern Europe had the lowest HM concentrations, whereas countries in Eastern and South-eastern Europe had the highest (Harmens *et al.* 2013, 2015). Undoubted, the passive biomonitoring with terrestrial mosses, constitutes a useful tool for the study of air quality and the atmospheric deposition of heavy metals, even though there exist some limitations (Aboal *et al.* 2010; Fernández *et al.* 2015). Some problem such as number and design of sampling sites, (Wolterbeek and Bode 1995; Aboal *et al.* 2006; Fernández *et al.* 2005; Amblard-Gross *et al.* 2004; Pesch *et al.* 2008) timing of surveys and moss species are important factors influencing the concentration of elements in moss tissues.

The following aims are addressed in this paper: (1) determination of trace elements concentrations in collected samples in altitudinal transect, (2) identify main phenomena based on correlations of trace elements concentrations and (3) describe interaction of main phenomena in context of altitude and the occurrence of road.

Material and Methods

The samples of *Dicranoweisia crispula* (Hredw.) Milde, were collected together with soil along road to The Stelvio Pass (Passo dello Stelvio) from Bormio

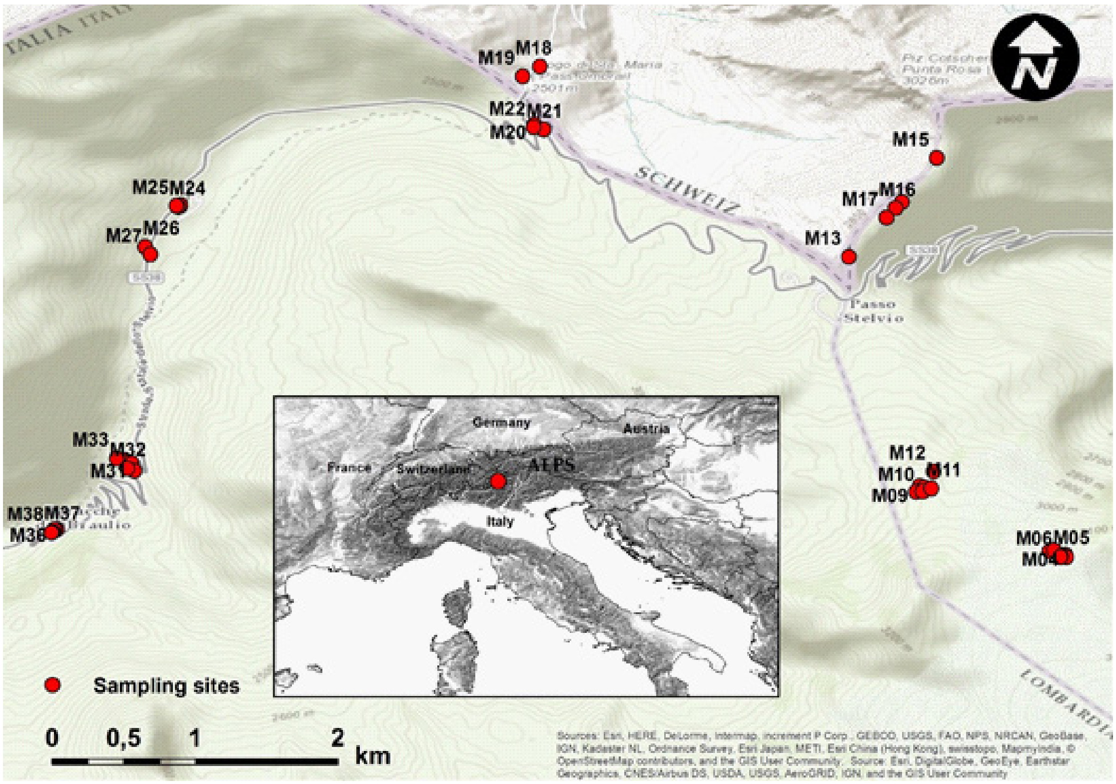

Fig. 1. Study area of the sampling sites where mosses *Dicranoweisia crispula* (Hredw.) Milde were collected together with soil (The Stelvio Pass, road from Bormio, northern Italy).

in northern Italy (Fig. 1) in altitudes from 2000 to 3171 m a.s.l., in 20th August 2015. The Stelvio Pass is the second highest paved mountain pass in the Alps. Localization data are described in Table 1. After the return to the laboratory, moss samples were gently cleaned from obvious soil particles, dried at 45° C for two days. During the drying process, the moisture of samples was analysed using laboratory scales. Each sample was carefully divided into two parts, the moss sample and sample of soil. Moss sample consisted only from green shoots and the soil sample was extracted from appropriates moss. After then moss and soil samples were homogenized in CryoMill (Retsch GmbH 2015) and analysed by X-ray fluorescence (Stephens and Calder 2004) using the hand-held XRF spectrometer DELTA CLASSIC (USA). The following elements were determined: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Sn, Sb, Ba, Pb. Measured data was joined to single data matrix of locality attributes and log-transformed.

For statistical analysis, the Statistica 12 (StatSoft, USA) was used. Nonparametric analysis (Mann-Whitney U test) and principal component analysis (PCA) was used to extract the potential relationships between the variables. First four principal components were tested with ANOVA to reveal the effect of the presence or absence of roads.

Results

By means of Shapiro-Wilk normality test all data do not have normal distribution except K, Cl in mosses and K, Ti in soil. A Mann-Whitney U test was used to compare the medians of the trace elements in areas without road ($n=16$) and areas by the road ($n=22$). Significant differences ($p<0.05$) between the median values of variables for two groups of data were in altitude, distance from road, concentration of K, Ti, Mn, Zn, Mo, Pb ($p=0{,}003$) in mosses and concentration of Mn, Ni, Mo, Hg in soil (Fig. 2).

Through nonparametric statistic for comparing two dependent samples we analysed concentrations of trace elements in mosses with concentrations in soil samples. Significant relations we observed in concentration of P in soil and Ni, Sn, As in mosses. Then concentration of Cl in soil with Ni, Zn in mosses, concentration of Pb in soil with Pb, Sn, Zn in mosses, concentration of S in soil with Sn in mosses, and concentration of Sn in soil was related with Cl, Ni, Zn, Sn, Pb in mosses.

From principal component analysis we determined four main phenomena. First of them, phenomenon PC1 (Table 2) represents the interaction of elements from background (soil) with elements stored in mosses in the context of altitude and localisation by the road (Fig. 3). Generally speaking, the level of trace elements concentration such as K Ti, Cr, Fe, Cu, Zn, As, Zr, Sn, Ba, and Pb increases with altitude in soil more than in mosses, except Fe and Cu.

Second phenomenon PC2 (Table 2, Fig. 4) describes situation of trace elements concentration in relation to road distance. Close to road are significant concentrations of Mn, As, Rb, Mo in mosses and P, K, As, Rb, Sn in soil. But further away from road are significant concentrations of S, Cl, Ca, Zn, Sr in mosses and Ca, Sr, Hg in soil.

Third phenomenon PC3 is a phenomenon of traffic pollution in lower altitude and by the roadside (Fig. 5) where trace elements such as Ti, Cr, Fe Zn, Sb together with Pb are deposited in soil more than in mosses.

Sample	Coordinates		Altitude [m a.s.l.]	Road distance [m]
	N	E		
M01	46 30.773	10 27.968	3170	2176
M02	46 30.779	10 27.986	3169	2178
M03	46 30.760	10 28.028	3171	2236
M04	46 30.753	10 28.033	3169	2249
M05	46 30.755	10 28.013	3169	2232
M06	46 30.753	10 28.009	3169	2234
M07	46 31.019	10 27.479	3023	1485
M08	46 31.014	10 27.496	3025	1502
M09	46 30.998	10 27.465	3022	1519
M10	46 30.998	10 27.488	3024	1527
M11	46 31.009	10 27.522	3024	1520
M12	46 31.073	10 27.531	3010	1413
M13	46 31.879	10 27.211	2830	240
M14	46 32.086	10 27.411	2849	712
M15	46 32.250	10 27.542	2873	1040
M16	46 32.062	10 27.387	2845	640
M17	46 32.027	10 27.352	2837	565
M18	46 32.593	10 26.043	2506	1
M19	46 32.557	10 25.978	2507	1
M20	46 32.376	10 26.021	2496	1
M21	46 32.357	10 26.057	2499	2
M22	46 32.365	10 26.019	2495	8
M23	46 32.073	10 24.687	2349	5
M24	46 32.067	10 24.677	2349	4
M25	46 32.071	10 24.671	2352	15
M26	46 31.917	10 24.552	2329	15
M27	46 31.888	10 24.571	2326	10
M28	46 31.115	10 24.474	2180	30
M29	46 31.108	10 24.489	2181	42
M30	46 31.104	10 24.503	2182	32
M31	46 31.075	10 24.512	2183	40
M32	46 31.085	10 24.485	2179	19
M33	46 31.123	10 24.445	2179	5
M34	46 30.856	10 24.217	2004	4
M35	46 30.856	10 24.211	2004	10
M36	46 30.856	10 24.209	2001	13
M37	46 30.846	10 24.201	2001	6
M38	46 30.844	10 24.199	2000	3

Table 1. Samples and characteristics of data collection.

Fourth phenomenon PC4 describes effect probably caused by cross-border transmission of trace elements. Concentration of lead is significantly higher in higher altitudes and further away from road (Fig. 6) mainly in soil. Conversely, concentrations of S, Ni, Cu, Mo in mosses and S in soil are higher in lower altitude.

Discussion

According the ICP-Vegetation manual (ICP Vegetation 2014), extensive surveys and sampling should be carried at a distance of least 300 m from main roads or urban and industrial areas, and at least 100 m from smaller roads and isolated houses (Frontasyeva *et al.* 2014). However, small scale biomonitoring based on the use of terrestrial mosses must be possible to verify differences in trace elements concentrations from areas

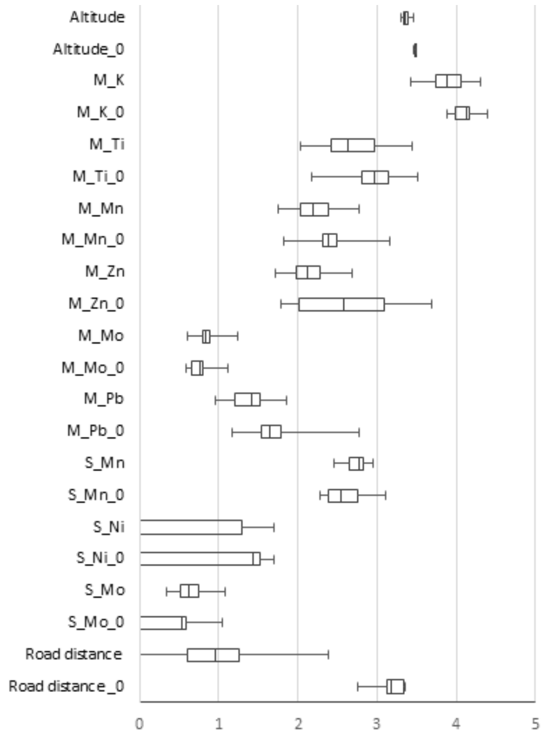

Fig. 2. Box plots of trace elements with significant differences (Mann-Whitney U test, p<0.05) between the median values (log transformed) of variables [Code: type of sample_ trace element_type of area; S - soil samples; M - mosses samples; areas without road - 0, (ex. Altitude_0)].

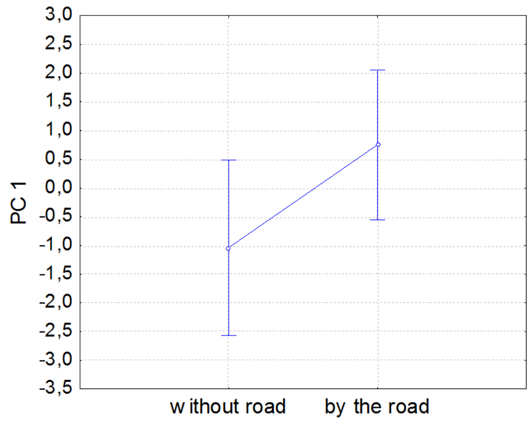

Fig. 3. The interaction of elements from soil with elements stored in mosses in the context of localisation by the road. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, F (4, 33) = 28.522, p = 0.00000.

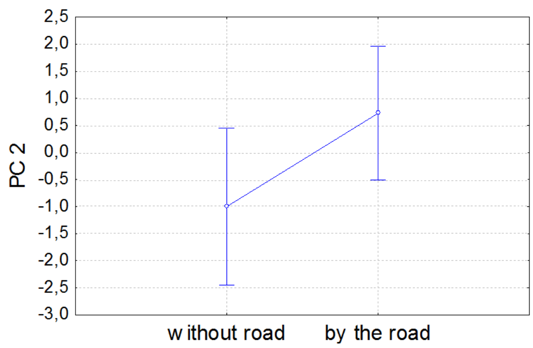

Fig. 4. The relation of trace elements stored in soil and mosses in the context of localisation by the road which are probably affected by acidification in higher altitude. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, F (4, 33) = 28.522, p = 0.00000.

Variables	PC1	PC2	PC3	PC4
Altitude [m a.s.l.]	-0.478	-0.297	-0.421	0.525
Road distance [m]	-0.334	-0.569	-0.426	0.483
M_S	-0.369	-0.457	0.038	-0.449
M_Cl	-0.347	-0.628	-0.037	-0.378
M_K	-0.658	0.204	-0.365	0.050
M_Ca	-0.035	-0.670	-0.451	-0.038
M_Ti	-0.772	-0.187	-0.295	-0.151
M_Cr	-0.552	0.342	-0.495	-0.266
M_Mn	-0.271	0.437	-0.129	0.145
M_Fe	-0.818	0.292	-0.328	-0.087
M_Ni	-0.251	-0.165	-0.291	-0.482
M_Cu	-0.643	0.090	-0.113	-0.548
M_Zn	-0.554	-0.429	0.353	0.367
M_As	-0.426	0.685	-0.290	0.288
M_Rb	-0.207	0.606	-0.358	0.306
M_Sr	-0.366	-0.547	-0.177	-0.084
M_Zr	-0.775	0.338	-0.387	-0.096
M_Mo	-0.033	0.555	-0.218	-0.430
M_Sn	-0.574	0.377	-0.033	0.051
M_Sb	0.069	-0.197	0.142	0.349
M_Ba	-0.708	-0.317	-0.231	-0.006
M_Pb	-0.439	-0.205	0.087	0.269
S_P	-0.411	0.444	0.071	0.011
S_S	-0.057	-0.197	0.495	-0.441
S_Cl	-0.207	-0.204	-0.022	0.254
S_K	-0.376	0.764	-0.101	0.251
S_Ca	0.088	-0.779	-0.498	0.075
S_Ti	-0.786	-0.070	0.418	-0.247
S_Cr	-0.710	-0.137	0.540	-0.039
S_Mn	-0.322	0.154	0.762	0.081
S_Fe	-0.607	0.299	0.461	-0.243
S_Ni	-0.714	-0.284	-0.033	0.041
S_Cu	-0.544	-0.180	0.165	0.132
S_Zn	-0.436	-0.345	0.568	0.363
S_As	-0.040	0.834	0.125	0.142
S_Se	0.085	-0.210	-0.238	0.144
S_Rb	-0.002	0.828	-0.139	0.316
S_Sr	-0.154	-0.649	-0.365	0.019
S_Zr	-0.663	0.226	0.219	-0.116
S_Mo	0.286	0.397	0.257	-0.179
S_Sn	-0.272	0.643	-0.297	0.158
S_Sb	-0.300	-0.222	0.438	0.368
S_Ba	-0.640	-0.181	0.220	-0.116
S_Hg	0.097	-0.418	-0.543	0.121
S_Pb	-0.346	-0.342	0.524	0.452
Eigenvalue	9.660	8.712	5.256	3.406
% Total variance	21.466	19.360	11.681	7.568
Cum. Eigenvalue	9.660	18.372	23.628	27.034
Cumulative %	21.466	40.826	52.506	60.075

Table 2. Principal component (PC) vectors (loadings), which indicate mutual interaction of trace elements in soil (S) and mosses (M). (Factor coordinates of the variables, based on correlations).

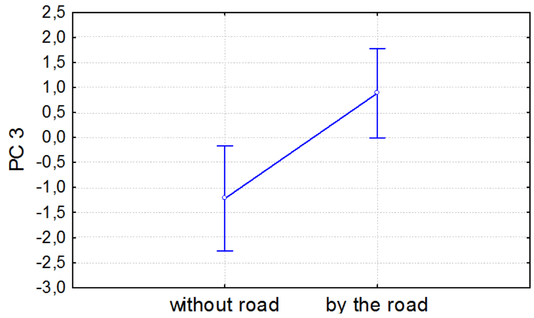

Fig. 5. The phenomenon of traffic pollution in lower altitude in the context of localisation by the road. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, F (4, 33) = 28.522, p = 0.00000.

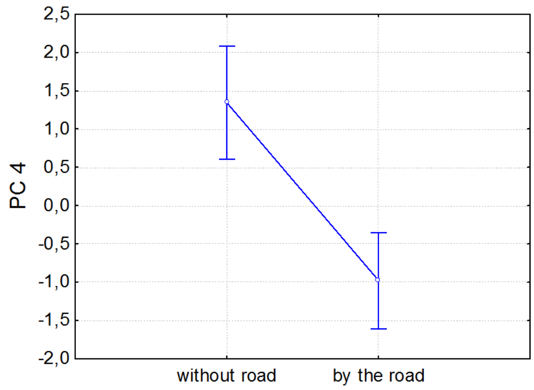

Fig. 6. The phenomenon of cross-border transmission of lead in higher altitudes in the context of localisation by the road. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, F (4, 33) = 28.522, p = 0.00000.

considered to be clean, and areas the surroundings of the focal point of the pollution (Fernández *et al.* 2007). Therefore, our data were localised in the places without road and in the places by the road. Samples from the places without road were at higher altitude due to elimination possible effect of traffic pollution. We selected only one moss species for this purpose. *Dicranoweisia crispula* (Mountain Pincushion) is an extremely variable species (Flowers 1956). It is a plant of montane rocks widespread mainly at higher elevations (Schofield 2007). It is bipolar species (Putzke and Pereira 2001) and one of the most dominant species of snow beds (Elvebakk 1984). *Dicranoweisia crispula* grows directly on rock or on damp gravel (Sean 2012), but also on burnt logs or downed wood in mid to advanced stages of decay (Clark 2012). We consider that this species could by a good species for indication of pollution in mountain regions. The process of trace elements accumulation to mosses depends on the nature of the contaminant and form in which it is emitted, then on physicochemical and biological processes and finally, all of these variables will depend on environmental factors such as precipitation, pH, salinity, temperature (Boquete *et al.* 2011). We suppose that the spatial variability of the trace elements concentration is very high due to heterogeneity of mountain condition and distances from anthropogenic sources of emission. Our results and detected higher concentrations of Pb in soil and mosses at higher altitude are consistent with the study of Zechmeister (1995) who analysed correlation between altitude and heavy metals deposition in the Alps. Our observed phenomena pointed to two sources of Pb in the environment. One is from transboundary emission and second is related to vehicular traffic. For possible sources of transboundary emission in Europe see e.g. Rühling (1994). Zechmeister *et al.* (2005) noted that the elements such as Sb, Mo, Cr Cu, As, Zn, and V can be regarded as indicators for traffic, Cd seems to be connected to other sources and elements like Ca or Al are predominantly an indicator for soil dust, as Ca is a frequent component of grit used on many roads during winter. The phenomenon of traffic pollution was significant in soil samples and closely connected except Pb also with other elements such as Ti, Cr, Mn, Fe, Zn, Sb. The phenomenon most likely related to touristic pressure as mentioned in study of Nascimbene *et al.* (2014). Schröder *et al.* (2010) found moderate to high correlations between cadmium and lead concentrations in mosses and modelled atmospheric deposition of these metals. In case of wet deposition some authors analysed trace element in Alpine snow. In general, mean trace elements concentrations found on the Eastern Alps (Gabrielli *et al.* 2006) were higher than those snow samples taken in the French western Alps (Veysseyre *et al.* 2001). In lower parts of Alps were concentrations found in the two studies are in good agreement. In medium altitudes concentrations found by Veysseyre *et al.* (2001) can be comparable to values determined in remote Greenland snow samples taken by Barbante *et al.* (2003). Dry deposition during dry weeks affecting the concentration in moss much more than the wet deposition (Berg and Steinnes 1997, Couto *et al.* 2004). If we have accepted some critical comments Reimann *et al.* (2001) and Aboal *et al.* (2010) about short-time concentration variability and Brown and Brumelis (1996) laboratory experiments, that the frequently presented interpretation that mosses progressively accumulate elements throughout their life is false. We have to accepting that mosses might then rather reflect the last rainfall instead of several years-accumulated depositions (Reimann *et al.* 1999). Which is in agreement with the results of Boquete *et al.* (2011) that the concentrations of the elements in the moss varied greatly within very short periods, and that the error associated with the temporal variability in the results was high. Notwithstanding the abundance of trace elements concentration, our data provide valuable information for improving the management and protection of mountain ecosystems in popular tourist areas.

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Short-term faunistic monitoring of four Sites of Community Importance (SCI) in the Pieniny National Park with suggestions of land management proposal

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Abstract. Sites of Community Importance (SCI) represent rare and endangered biotopes of the Natura 2000 network. The objective of SCI protection and management is to keep their landscape and ecological function as well as their biological diversity in good condition. In our study, four SCI in the buffer zone of Pieniny National Park, Slovakia were investigated. We surveyed selected aquatic and terrestrial invertebrates during a short-term period in early summer 2015, in the water and water dependent biotopes and their coastal zones. The consistent reconnaissance of the actual status of sampling sites was important, as we confirmed the presence of several rare, endangered and Natura Directive species of flies, mayflies and dragonflies as well as terrestrial spiders and ground beetles. Application of the management proposal, which we suggested with respect to the bionomy and habitat requirements of present protected species, should lead to improvement of actual conditions of the monitored SCI.

Key words: terrestrial, aquatic, invertebrates, SCI, PIENAP, management

Introduction

Sites of Community Importance (SCI) as a part of Special Areas of Conservation (SAC) were designated to meet specific conservation objectives achievable by appropriate conservation measures. They also provide a wide range of provisioning, regulation and socio-cultural ecosystem services (IEEP 2002; Schweppe-Kraft 2008; Kettunen *et al.* 2009) dependent on particular species, groups of species, habitat types, vegetation structures or land cover (Bastian 2013).

Natura 2000, an EU-wide network of nature protection areas established under the 1992 Habitats Directive was designed to achieve the aim of the European Community, i.e. "to halt the loss of biodiversity and the degradation of ecosystem services in the EU by 2020, restore them as far as feasible, while stepping up the EU contribution to averting global biodiversity loss" (Viceníková and Polák 2003; COM 2011) and to assure the long-term survival of Europe's most valuable and threatened species and habitats. Its establishment also fulfills a Community obligation under the UN Convention on Biological Diversity. The Natura 2000 network includes nature reserves, however, most of the included land is privately owned and the emphasis is on ensuring its ecological and economically sustainable management.

Our study was carried out according to the framework of objectives of the "Development of management plans for selected sites included in the Natura 2000". The project was focused on a biodiversity inventory of selected invertebrate groups including macrozoobenthos, Diptera and terrestrial Araneae and Coleoptera and the development of a management proposal to maintain the status of four SCI in the Pieniny National Park protective zone: Veľké Osturnianske jazero lake, Malé Osturnianske jazerá lakes, Jarabinský prielom gorge and Plavečské štrkoviská gravel deposit.

In general, the area of Pieniny National Park is faunistically well studied. Information about the fauna and flora of this area were published by Vološčuk (1992) and Razowski (2000), and several papers focusing on the particular animal groups from this area were also published (e. g Arachnida - Svatoň 1990; Coleoptera - Jászay 1997; 1999; 2001; 2007; aquatic invertebrates - Manko and Zaťovičová 2006; Lepidoptera - Panigaj 2008). On the other hand, some taxa and particular areas and sites remained almost ignored. With the exception of the paper published by Šácha (2010), including data on fauna of the Odonata in the Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes, there are no other published data on the invertebrate fauna of our researched sites.

The targets of SCI protection are both the Natura Directive species as well as habitat types. At the SCI location we studied, dominant aquatic species included *Bombina variegata* (Linnaeus, 1758), *Castor fiber* (Linnaeus, 1758), *Hamatocaulis vernicosus* (Mitt.) Hedenäs, *Lissotriton montandoni* (Boulenger, 1880) (Veľké Osturnianske jazero lake, Malé Osturnianské jazerá lakes) or *Cottus gobio* (Linnaeus, 1758), *Hucho hucho* (Linnaeus, 1758), *Lutra lutra* (Linnaeus, 1758), and *Castor fiber* (Linnaeus, 1758) (Plavečské štrkoviská gravel deposit). Interesting plants including bogbean (*Menyanthes trifoliata* L.), marsh lousewort (*Pedicularis palustris* L.), lesser tussock sedge (*Carex diandra* Schrank) and round leaved sundew (*Drosera rotundifolia* L.) could be also found here. The targets of habitat protection are mostly water dependent habitats, including natural eutrophic and dystrophic lakes and ponds, transition mires and quaking bogs, petrifying springs, alkaline fens and species-rich *Nardus* grasslands at Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes, alpine rivers with ligneous vegetation (*Salix eleagnos* Scop.), species-rich *Nardus* grasslands, hydrophilous tall herb fringe communities of plains and alkaline fens at Jarabinský prielom gorge and alpine rivers with ligneous vegetation at Plavečské štrkoviská gravel deposit.

Therefore the aim of our study was to:
a) create a checklist and characterise assemblages of selected groups of aquatic and terrestrial invertebrates that contribute to the knowledge of invertebrate fauna of Pieniny National Park from four stands representing Sites of Community Importance (SCI) belonging to the Natura 2000 network, (Veľké Osturnianske jazero lake, Malé Osturnianske jazerá lakes, Jarabinský prielom gorge and Plavečské štrkoviská gravel deposit)
b) environmental reconnaissance to aid development of a management proposal with the objective of maintaining the status of of biotopes and their services with regard to the present taxa of aquatic and terrestrial invertebrates.

Material and Methods

Sampling sites

The sampling of terrestrial and aquatic invertebrates was carried out during the 2015 at four sampling sites within localities representing Sites of Community Importance (SCI), belonging to NATURA 2000 network (Fig. 1). The short characterisation of the sampling sites are as follows:

Veľké Osturnianske jazero lake, registered under the code SKUEV0334 (Natura 2000 - Standard data form); N 49° 20.49', E 0° 13.204', elevation 815 m a. s. l., area of water surface 0.25ha, total SCI area 45.51ha. The site lies within the western part of the buffer zone of Pieniny National Park, in near vicinity of Osturňa village. The lake is through-flow, supplied by surface flows as well as underground springs. The coastal zone is dominated by sparsely occurring shrubs and pioneer vegetation (*Salix* sp.), and monocoenose of *Picea abies* (L.) (Vološčuk 1992; Lacika and Ondrejka 2009; Košický and Ivanič 2011). *Malé Osturnianske jazerá lakes*, registered under the code SKUEV0335 (Natura 2000 - Standard data form); N 49° 20.254´, E 20° 12.348´, elevation 883 m a. s. l., area of water surface 0.06ha, total SCI area 6.465ha. The site is situated within the buffer zone of the Pieniny National Park, near the border with Poland, in south-west of Veľké Osturnianske jazero lake. The site was designed as a nature reserve in 1984. It features very rich aquatic and wetland communities well adapted to the fluctuating water level. Local aquatic and wetland communities are mostly surrounded by meadows and forests. The site is also prone to landslides following prolonged periods of rain (Vološčuk 1992).

Jarabinský prielom gorge, registered under the code SKUEV0339 (Natura 2000 - Standard data form); N 49° 20.864', E 20° 38.899', elevation 652 m a. s. l.. Jarabinský prielom gorge makes up part of the SCI Pieninské bradlá cliffs – a larger area of limestone cliffs, belonging to the geomorphological unit Ľubovnianska vrchovina highlands and Pieniny (Vološčuk 1992; Košický and Ivanič 2011). It is a water shaped limestone gorge with five cascades and large potholes (5m in diameter, 3m deep) located 630 - 700 m a. s. l. and covering an area of 5.55 ha. The sampling site lies north of Jarabina village in the near vicinity of the local stone quarry. Malý Lipník brook flow through this area, creating entire limestone desks, cascades and waterfalls. Its coastal zone is dominated by *Acer* sp., *Abies alb*a (Mill.) and *Picea abies* (L.) and has forest character.

Plavečské štrkoviská gravel deposit, registered under the code SKUV0338 (Natura 2000 - Standard data form); N 49° 15.534', E 20° 50.872', elevation 480 m a. s. l., total SCI area 66.24ha. This gravel deposit was created through natural reclamation following gravel-sands mining. The stand is located within the meander of the Poprad River, in the vicinity of Plaveč village, within the geomorphological unit of Spišsko – šarišské medzihorie. The area includes extensive water planes, wetlands, flooded forests, the part of Ľubotínka brook and Poprad River (Košický and Ivanič 2011).

Material collection and identification

Terrestrial and aquatic invertebrates were sampled using standard methods on May 14th, June 3rd, and June 17th of 2015. The focus was on the capture of the maximum spectrum of taxa/species possible, rather than on quantitative collection, and therefore, we focused on material sampling possible within the widest spectrum of present microhabitats. Thus, abundance is expressed as an absolute number, not counted as activity abundance.

Aquatic invertebrates were collected with a hydro biological net (0.35mm density) using the "kicking sampling" method. Samples were taken from all presented microhabitats, including the whole flow profile, to a flow depth of 0.75 meters by deep flowing or standing waters. Specimens from submerged wood were hand-collected.

The adults of aquatic invertebrates and flying insects were collected using sweep netting.

Terrestrial invertebrates were collected using five pitfall traps with a 4% formaldehyde water so-

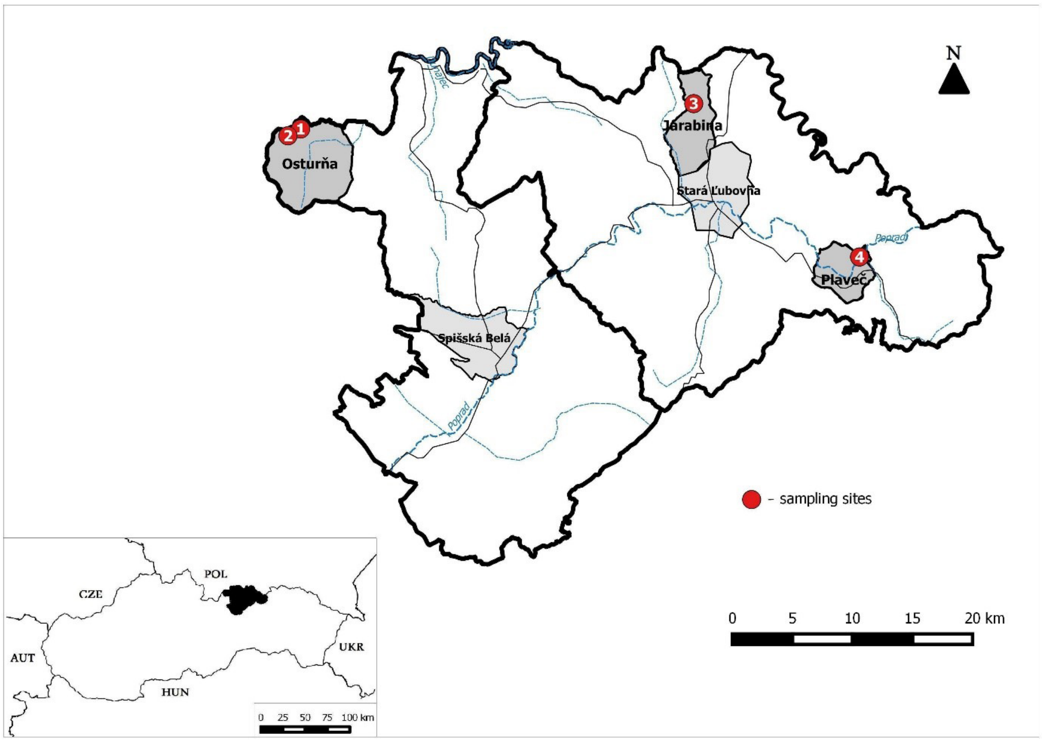

Fig. 1. Map with the position of sampling sites in the northeastern part of Slovakia, Pieninský National Park, 1-Veľké Osturnianske jazero lake, 2-Malé Osturnianske jazerá lakes, 3-Jarabinský prielom gorge, 4-Plavečské štrkoviská gravel deposit.

lution, set in a line within the coastal zone of the studied water planes, between May 14 and June 17, 2015. Spiders were also supplemented with individual collection and vegetation sweeping.

Invertebrates were manually sorted and identified up to the order level.

Material of epigeic spiders (Araneae), ground beetles (Coleoptera: Carabidae), dipterans (Diptera), mayflies (Ephemeroptera), caddisflies (Trichoptera), stoneflies (Plecoptera), dragonflies (Odonata) and aquatic true bugs (Heteroptera) were identified to the species level using appropriate determination keys (Kis 1974; Rozkošný 1980; Heimer and Nentwig 1991; Hůrka 1996; 2005; Nilsson 1997; Waringer and Graf 1997; Bauernfeind and Humpesch 2001; Van Veen 2004; Bauernfeind and Soldán 2012; Krno 2013; Oosterbroek 2015).

Abundance is expressed in absolute numbers. A checklist of determined species of epigeic spiders, ground beetles, dipterans, mayflies, caddisflies, stoneflies and dragonflies (Odonata) is introduced in Appendix A. The rest of identified invertebrate species is introduced in Appendix B.

Material was preserved in ethanol and stored by individual authors (Araneae – Zuzana Krumpálová; terrestrial Coleoptera - Beáta Baranová; Diptera - Jozef Oboňa; Ephemeroptera, Trichoptera - Ľuboš Hrivniak; Plecoptera – Peter Manko; Odonata - Zuzana Matúšová; aquatic true bugs - Barbora Reduciendo Klementová).

We characterised assemblages from their structural view-point and recorded the presence of valuable and indicator species. Software (vers. 3.10 - Hammer et al. 2001) was used to calculate assemblage diversity and evenness.

The development of management proposals

We conducted consistent reconnaissance of the actual status of study stands, observed and evaluated the presence of pioneer vegetation, non-indigenous plant species and estimated successional stage. Additionally, we assessed potential local anthropogenic interventions and indirect human impact.

We evaluated assemblage structure and recorded the presence of rare, endangered and Natura Directive species and discussed management proposal suggestions that aim to induce or maintain the status, natural character as well as the landscape and ecological function of the SCI, respecting habitat and bionomy requirements of present taxa with lay emphasis on protected species.

According to types of studied habitats, our management suggestions are focused mainly on suitable water regime maintenance including:

- water supply improvement and retention, sufficient water level maintenance (standing water)

- avoidance of vulnerable modification of flow regime i.e. anthropogenic intervention into the natural processes forming riverbanks and riverbed, respecting natural or near-to-nature flow regimes and natural hydromorphology (flowing waters)

- creating a functional, structurally diversified riparian buffer zones which will preserve and improve good conditions for aquatic invertebrates and other terrestrial taxa related to aquatic biotopes (Bunn and Arthington 2002; Postel and Richter 2012; Kuglerova et al. 2014);

In the last phase, we finalized our land management proposals for the State Nature Conservancy of the Slovak Republic (Administration of the Pieniny National Park).

Results and Discussion

Assemblage characterisation

Spiders (Araneae)

Overall, 6 species belong to the one of the categories of threatened species (following Gajdoš *et al.* 1999), *Mioxena blanda* (Simon, 1884) – LR (nt), *Alopecosa striatipes* (C.L.Koch, 1839) and *Hahnia helveola* (Simon, 1875) – LR (nc), *Alopecosa pinetorum* (Thorell, 1856) and *Ozyptila rauda* (Simon, 1875) – VU, *Tapinocyba biscissa* (O.P.-Cambridge, 1872) – DD. Ground-living spider assemblages of Veľké Osturnianske jazero lake, Malé Osturnianske jazerá lakes and Jarabinský prielom gorge riparian zones were characterised by a high proportion of forest species with preference to wet and shady habitats (predominance of - *Callobius claustrarius* (Hahn, 1831) and *Coelotes inermis* (L. Koch, 1855)) that reflect habitat conditions and indicate non-disturbed habitats. The proportion of hygrophilous and hemihygrophilous species was more than 85% of all specimens. However, a higher abundance of eurytopic species *Pardosa lugubris* (Walckenaer, 1802) signalled changes in the hydrological regime at these study sites. This means that these habitats continually dried up, confirming the findings of xerophilous spider species there (mainly at Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes). The spider *Pardosa monticola* (Clerck, 1757), typical in dry open habitats was predominant at the drier, human influenced sampling site of the Plavečské štrkoviská gravel deposit. Spider assemblage was characterised by a high number of species with a single, unbalanced presence. Additionally, we confirmed the presence of the vulnerable species *O. rauda*, as well as the less at risk species *H. helveola* and *A. striatipes* or *T. biscissa* in this location.

Ground beetles (Coleoptera: Carabidae)

Ground beetle assemblages at Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes riparian zones were dominated by large wingless forest species with a preference for wet and shady habitats what in accordance with this stand's characteristic environment. We did not confirm the presence of any of Natura Directive species, though the presence of *Carabus auronitens escheri* (Palliardi, 1825) as well as *Pterostichus aethiops* (Panzer, 1797) and *Pterostichus foveolatus* (Duftschmid, 1812) was interesting. The same structure of ground beetle assemblage was confirmed for the Jarabinský prielom gorge, where the vulnerable red list species protected by Natura 2000, *Carabus variolosus* (Fabricius, 1787) was found. This species inhabits mountain streams and banks and indicates that non-disturbed habitats sensitively react to human disturbances (Holecová and Franc 2001; Veselý *et al.* 2005; Matern *et al.* 2007; 2008). At Plavečské štrkoviská gravel deposit, the ground beetle community was dominated by small, macropterous species typical in dry, open habitats without special requirements for environmental conditions. We did not confirm the presence of any of the Natura 2000 protected species, but we found the two interesting species of note - *Agonum viduum* (Panzer, 1796) and *Chlaenius tibialis* (Dejean, 1826) - at this location (Table 1).

Flies (Diptera)

The presence of faunistically interesting species of the Syrphidae family, *Anasimyia lineata* (Fabricius, 1787) and *Parhelophilus versicolor* (Fabricius, 1794) was recorded at Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes. Both species are rare and occur mostly on the edges of aquatic ecosystems (Speight 2011). In the Jarabinský prielom gorge, we confirmed the presence of extremely rare European species from the family Empididae, *Chelifera aperticauda* (Collin, 1927) (Oboňa *et al.* 2016). In the Plavečské štrkoviská gravel deposit, the presence of species from the family Limoniidae, *Arctoconopa melampodia* (Loew, 1873), *Hexatoma (Hexatoma) bicolor* (Meigen, 1818) and *Hexatoma (Hexatoma) fuscipennis* (Curtis, 1836) were recorded. These species inhabit sand and gravel banks of

	Site n. 1	Site n. 2	Site n. 3	Site n. 4
Spiders				
Taxonomic richness (species)	14	27	15	33
Absolute abundance	116	191	54	296
Diversity H´	1.81	2.26	2.12	2.14
Evenness_e^H/S	0.44	0.36	0.55	0.26
Carabidae species				
Taxonomic richness	11	5	19	24
Absolute abundance	54	38	141	114
Diversity (H)	2.01	1.04	2.21	2.58
Evenness (J)	0.84	0.65	0.75	0.81

Abbreviations and notes: Site n. 1 – Veľké Osturnianske jazero lake, Site n. 2 - Malé Osturnianske jazerá lakes, Site n. 3-Jarabinský prielom gorge, Site n. 4 – Plavečské štrkoviská gravel deposit

Table 1. Characterisation of spiders and ground beetles assemblages of particular sampling sites.

the larger streams and are very sensitive to the destruction of the natural structure of the river as well as habitat pollution (e.g. Podeniene 2002). We also recorded the Slovakian vulnerable species *Atherix ibis* (Fabricius, 1798) from family Athericidae (Jedlička and Stloukalová 2001) at the site (Table 2).

Mayflies (Ephemeroptera)

Cloeon dipterum (L.), widespread across Europe and Asia was the only mayfly species found at Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes. Despite the absence of protected species, Jarabinský prielom gorge was characterised by relatively rare mayfly community structure, and instead dominated by rheobionts and rheophilic species of rhithral (mainly metarhithral), preferring stony substrate, feeding as grazers – scrapers and gatherers – and collectors. Such a community is typical for colline and submontane central European streams, and is characteristic of the cliff zones. Two Slovakian endangered species (Deván 2001) sensitive to pollution and change in environmental conditions (Bauernfeind and Soldán 2012) were found in the Plavečské štrkoviská gravel deposit: *Ecdyonurus insignis* (Eaton, 1870) – a rheophilous species of hyporhithral and metarhithral sections of rivers with stony bottom (Derka 2003) and *Oligoneuriella rhenana* (Imhoff, 1852) – a stenotopic filter-feeder, that inhabits montane, sub-montane and occasionally lowland rivers. Their life cycle is short (May to August) (Haybach 2006), and larvae occur in places with a strong current and stony bottom (Elpers and Tomka 1995; Bauernfeind and Soldán 2012). Concerning whole assemblages, a high occurrence of species with a lithal, phytal and pelal preference indicates relatively high microhabitat diversity as the community is supplemented by species with preference to psamal, akal and other microhabitats. The abundance of grazers, - scrapers and gatherers – collectors and the presence of passive filter feeders indicates the presence of fine particular organic matter in the lentic micro- and mesohabitats (Table 2).

Stoneflies (Plecoptera)

Stonefly species found at Veľké Osturnianské jazero lake, belonging to the crenal and rhithral rheophilic fauna and with preference to lithal and phytal are more typical in outlet streams than in lake habitats (f.e *Nemoura cinerea* Retzius, 1783). No stoneflies species was found at Malé Osturnianske jazerá lakes. The Plecoptera assemblage of the Jarabinský prielom gorge consisted of seven species with preference to lithal and forming rheophilic stoneflies conenoses of epirhithral. Plecopterofauna of the Plavečské štrkoviská gravel deposit was lowly taxonomically diversified, but yet relatively highly diversified from the microhabitat preference point of view. It was dominated by species preferring akal, phytal and particular organic matter, which indicates high substrate and microhabitat diversity. Although we did not find any rare or especially interesting species, the presence of the Plecoptera order in itself indicates good environmental conditions and value of the studied aquatic biotopes (Table 2).

Caddisflies (Trichoptera)

The dominant caddisfly species at Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes, limnobiont *Limnephilus stigma* (Curtis, 1884) usually inhabits the littoral zone of standing waters or potamal zones of rivers (Zaťovičová and Novikmec 2003). The caddisfly assemblage of Jarabinský pri-

	Site n. 1	Site n. 2	Site n. 3	Site n. 4
Flies				
Taxonomic richness (species)	14	10	20	15
Absolute abundance	86	62	54	76
Number of families	5	5	6	3
Mayflies				
Taxonomic richness (species)	1	1	15	19
Species absolute abundance	101	78	741	294
Number of families	1	1	6	7
Stoneflies				
Taxonomic richness (species)	4	-	7	5
Absolute abundance	17	-	31	59
Number of families	2	-	3	5
Caddisflies				
Taxonomic richness (species)	3	3	14	13
Absolute abundance	20	22	159	56
Number of families	1	1	9	7

Abbreviations and notes: Site n. 1 – Veľké Osturnianske jazero lake, Site n. 2 - Malé Osturnianske jazerá lakes, Site n. 3-Jarabinský prielom gorge, Site n. 4 – Plavečské štrkoviská gravel deposit

Table 2. Characterisation of flies, mayflies, stoneflies and caddisflies assemblages of particular sampling sites.

elom gorge was dominated by rheophilous species which inhabit psammal, akal, lithal, phytal and particulate organic matter. This community is typical of epirhithral – metarhithral zones of montane - colline sections of streams and rivers. Most of these species belong to grazer-scraper and shredder feeding types, and some of them (e.g. *Hydropsyche saxonica* (Mclachlan, 1884)) are particularly sensitive to pollution (Higler and Tolkamp 1982; Móra *et al.* 2004). The caddisfly assemblage of Plavečské štrkoviská gravel deposit, typical for the metarhithral section of submontane – colline rivers, was dominated by rheobiontic and rheophilic species which inhabit akal, lital and phytal zones. In general, we did not recorded any rare or endangered Trichoptera species (Table 2).

Dragonflies (Odonata)

A rare (Šácha 2010) and vulnerable (David 2001) dragonfly Coenagrion hastulatum (Charpentier, 1825) was found at Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes. The species inhabits littoral zones of peatlands, and dystrophic and oligotrophic lakes (with low pH values) with a dense occurrence of macrophytes (*Carex* spp.) and is very sensitive to habitat degradation, including the meliorations of wetlands, exploitation of peat, pollution, damage of littoral zones as well as the natural succession of ponds (Dolný *et al.* 2007). At the Plavečské štrkoviská gravel deposit (its part Poprad River), we recorded the rheophilic species *Onychogomphus forcipatus* (Linnaeus, 1758), belonging to the vulnerable species list in Slovakia (David 2001). This species inhabits colline and submontane sections of rivers, with a preference for gravel and sandy bottoms (Bulánková 2003; Petrovičová and David 2013) and is sensitive to the destruction of natural bank structure, regulation of rivers, and the exploitation of gravel (Dolný *et al.* 2007; Petrovičová and David 2013).

Sites characterisation and land management proposal

Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes

The Lakes area was of a larger scale in the past, as evidenced by their boggy surroundings. Reduction of the lake habitats can even be observed nowadays as the lakes are being gradually overgrown with vegetation spreading from adjacent forest stands and abandoned meadows. The changes in monthly precipitation (Hlavcova and Cunderlik 1998) and in snow cover durations and snow-melt related water supply (Vojtek *et al.* 2003) contribute to their drying-out. Lakes are in a state of desiccation, and the process seems to be irreversible. Thus, the most valuable part of this site - aquatic and wetland communities- are under threat. In general, the site is not exposed to direct anthropogenic disturbances.

Suggested management proposals that encourage desiccation mitigation include:

- Place opened, dewatering channels against the road direction. These channels would drain rain water in the direction of the lake;

- In the forest which separates road and lakes to clear cut narrow corridors to prevent encourage the flow of water toward the lake;

- Both sites could excavate several deep holes able to maintain a higher amount of precipitation during rainy season. Such artificial microhabitats could play a pivotal role as refuges for local aquatic faunal communities during the dry period;

- Remove succession pioneer vegetation and part of pine monoculture within the lakes coastal zone, clear-cut of self-seeding trees and shrubs to create small coastal islands and broaden the coastal zone. This could help to increase microhabitat heterogeneity in the area as well as delay the source of pioneer vegetation;

- Build up a dam in the lowest point of both lakes, that could help to keep the water in the lake bodies. The biomass harvested in the proposed step above could be used to accomplish this.

Jarabinský prielom gorge

This site is an enclave of water canyon adjoined by wide xerothermic meadows, grazing land and rocks, creating specific conditions for the existence of isolated hygrophilous invertebrates (e.g. ground living spiders or beetles). The monitored area is used for trekking. Several maintenance projects including fallen tree removal as well as an entry path at the upper meadow are in progress. The stone quarry adjacent at the bottom of the gorge increases dust particulate in the air, while the surrounding upper localised meadows are used for cattle or sheep grazing. However, in general, the site is not exposed to a large degree anthropogenic disturbances. Suggested management proposal are as follows:

- Maintain natural character of the coastal zone including vegetation coverage;

- Continually manage the meadows and grazing land;

- Respect natural formation/succession processes of brook-basin and surrounding habitats;

- Avoid flow modifications such as flow regulation; and

- Minimise risk of organic or chemical pollution.

Plavečské štrkoviská gravel deposit

When compared to the others, this site is exposed to a higher degree of anthropogenic impact. Because part of the gravel deposit is used for a fishery, there is greater human and vehicle activity, and regular harvesting occurs in grassy areas. Habitats surrounding the water reservoir are relatively monotonous from a structureal point of view. The more structurally diversified habitats are those within the Poprad River coastal zone. During the monitoring season, the stream-bed of Ľubotínka Brook was excavating using heavy machinery. As a result, coastal zone vegetation was destroyed on the left river bank for about three kilometers.

Suggested management arrangements are as follows:

- Regulate and clearly eliminate human and agro-activities;

- Avoid use of flow modification, including regulation and modification of the hydrological regime;

- Remove a portion of the bank's woody vegetation which has overgrown sand and gravel banks to create 'un-treed islands'.

Conclusions

Results obtained within our study indicate that short-term faunistic monitoring including several invertebrate groups could paint an adequate portrait of actual SCI status and lead to the development of necessary management actions. The fact that short-term monitoring is more respectful of local faunal communities paired with its cost effectiveness should not be ignored. Within the Veľké Osturnianske jazero lake and Malé Osturnianske jazerá lakes region we recommended active management interventions necessary to decelerate the unfavorable process of desiccation, while within the Jarabinský prielom gorge and Plavečské štrkoviská gravel deposit we recommend minimal intervention, and maintain that a "hands-free" management approach would be the best fir. Individual accession to management of a particular SCI is necessary to ensure their landscape and ecological function as well as their biological diversity and resilience.

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Appendix A

Checklist of determined terrestrial spiders, ground beetles, flies, mayflies, stoneflies, caddishflies and dragonflies species and number of encaptured specimens found at four SCI sites, Veľké Osturnianske jazero lake, Male Osturnianske jazerá lakes, Jarabinský prielom gorge and Plavečské štrkoviská gravel deposit, Pieniny National Park, in the early summer 2015. Abbreviations and notes: Site n. 1-Veľké Osturnianske jazero lake, Site n. 2-Malé Osturnianske jazerá lakes, Site n. 3-Jarabinský prielom gorge, Site n. 4-Plavečské štrkoviská gravel deposit.

		Site n. 1	**Site n. 2**	**Site n. 3**	**Site n. 4**
Araneae					
Dysderidae	*Harpactea rubicunda* (C.L.Koch, 1839)				1
Theridiidae	*Theridion pictum* (Walckenaer, 1802)				2
Linyphiidae	*Ceratinella brevis* (Wider, 1834)	2			
	Diplocephalus cristatus (Blackwall, 1833)	3			1
	Diplocephalus helleri (L.Koch, 1869)	1			
	Entelecara acuminata (Wider, 1834)		2		
	Erigone dentipalpis (Wider, 1834)			1	2
	Kaestneria dorsalis (Wider, 1834)				3
	Mughiphantes mughi (Fickert, 1875)		3		
	Tenuiphantes mengei (Kulczynski, 1887)		1		
	Tenuiphantes tenebricola (Wider, 1834)	3		1	
	Maso sundevalli (Westring, 1851)		1		
	Mioxena blanda (Simon, 1884)		1		
	Oedothorax retusus (Westring, 1851)				2
	Syedra gracilis (Menge, 1869)		1		
	Tapinocyba biscissa (O.P.-Cambridge, 1872)		2		3
	Walckenaeria atrotibialis (O.P.-Cambridge, 1878)			1	1
	Walckenaeria cucullata (C.L.Koch, 1836)		1		
	Walckenaeria mitrata (Menge, 1868)	1			
	Walckenaeria obtusa (Blackwall, 1836)				1
Tetragnathidae	*Pachygnatha degeeri* (Sundevall, 1830)				3
	Pachygnatha listeri (Sundevall, 1830)	1			
	Tetragnatha extensa (Linnaeus, 1758)				3
	Tetragnatha montana (Simon, 1874)				2
	Tetragnatha pinicola (L.Koch, 1870)		1		
Araneidae	*Araneus diadematus* (Clerck, 1757)				1
	Araneus marmoreus (Clerck, 1757)		1		
	Araniella cucurbitina (Clerck, 1757)		3		
	Hypsosinga albovittata (Westring, 1851)				2
	Larinioides cornutus (Clerck, 1757)			1	
	Singa nitidula (C.L.Koch, 1845)				1
Lycosidae	*Alopecosa cuneata* (Clerck, 1757)		1		
	Alopecosa pinetorum (Thorell, 1856)			5	
	Alopecosa striatipes (C.L.Koch, 1839)				2
	Alopecosa trabalis (Clerck, 1757)		14		
	Pardosa amentata (Clerck, 1757)	13	8		32
	Pardosa monticola (Clerck, 1757)				141
	Pardosa prativaga (L.Koch, 1870)				20
	Pardosa lugubris (Walckenaer, 1802)	17	20	2	
	Pardosa sp.			5	
	Piratula hygrophila (Thorell, 1872)			3	
	Trochosa ruricola (De Geer, 1778)				22

	Trochosa spinipalpis (F.O.P.-Cambridge, 1895)	3	7		
	Trochosa terricola (Thorell, 1856)		14	1	
Pisauridae	*Pisaura mirabilis* (Clerck, 1757)				1
Agelenidae	*Tegenaria campestris* (C.L.Koch, 1834)		2	2	
Hahniidae	*Hahnia helveola* (Simon, 1875)				5
	Hahnia pusilla (C.L.Koch, 1841)	1			17
Amaurobiidae	*Callobius claustrarius* (Hahn, 1831)	50	75	19	
	Coelotes inermis (L.Koch, 1855)	18	20	10	
Liocranidae	*Agroeca brunnea* (Blackwall, 1833)	2			
	Liocranoeca striata (Kulczynski, 1882)	1	1		
	Scotina celans (Blackwall, 1841)				1
Phrurolitidae	*Phrurolithus festivus* (C.L.Koch, 1835)				9
Clubionidae	*Clubiona lutescens* (Westring, 1851)			1	
	Clubiona stagnatilis (Kulczynski, 1897)				1
	Clubiona sp.			1	
Gnaphosidae	*Drassodes pubescens* (Thorell, 1856)				5
	Zelotes subterraneus (C.L.Koch, 1833)		1	1	
Philodromidae	*Philodromus collinus* (C.L.Koch, 1835)		6		
Thomisidae	*Diaea dorsata* (Fabricius, 1777)		1		2
	Ozyptila praticola (C.L.Koch, 1837)		1		1
	Ozyptila rauda (Simon, 1875)				2
	Ozyptila simplex (O.P.-Cambridge, 1862)				2
	Xysticus cristatus (Clerck, 1757)		2		4
	Xysticus ulmi (Hahn, 1831)				1
Salticidae	*Ballus chalybeius* (Walckenaer, 1802)		1		
Coleoptera					
Carabidae	*Abax carinatus* (Duftschmid, 1812)			2	
	Abax parallelepipedus (Piller & Mitterpacher, 1783)			55	
	Abax parallelus (Duftschmid, 1812)			14	
	Agonum muelleri (Herbst, 1784)				2
	Agonum viduum (Panzer, 1797)				1
	Amara erratica (Duftschmid, 1812)				15
	Amara spp. (Bonelli, 1810)				4
	Anysodactylus binotatus (Fabricius, 1787)				1
	Asaphidion pallipes (Duftschmid, 1812)				3
	Bembidion guttula (Fabricius, 1779)				6
	Bembidion lampros (Herbst, 1784)				19
	Bembidion assimile (Gyllenhal, 1810)				2
	Calathus fuscipes (Goeze, 1777)				3
	Carabus auronitens escheri (Palliardi, 1825)	7		1	
	Carabus glabratus (Paykull, 1790)	8	2	12	
	Carabus granulatus (Linnaeus, 1758)			1	1
	Carabus hortensis (Linnaeus, 1758)			1	
	Carabus variolous (Fabricius, 1787)			2	
	Carabus violaceus (Linnaeus, 1758)	5		1	1
	Cymindis humeralis (Fourcroy, 1785)				1
	Harpalus afinis (Schrank 1781)				10
	Harpalus latus (Linnaeus, 1758)	2	3	2	1
	Harpalus luteicornis (Duftschmid, 1812)				1
	Chlaenius tibialis (Dejean, 1826)				2
	Molops piceus (Panzer, 1793)	18	25	8	

	Nebria brevicolis (Fabricius, 1792)	2	1		24
	Ophonus azureus (Fabricius, 1792)				1
	Platynus assimilis (Paykull, 1790)			7	
	Poecilus cupreus (Linnaeus, 1758)				4
	Poecilus versicolor (Sturm, 1824)				7
	Pterostichus aethiops (Panzer, 1797)	2	7	12	
	Pterostichus anthracinus (Illiger, 1798)				1
	Pterostichus burmeisteri (Illiger, 1798)	6		3	
	Pterostichus foveolatus (Duftschmid, 1812)			4	
	Pterostichus melanarius (Illiger, 1798)			1	3
	Pterostichus niger (Schaller, 1783)	1		3	
	Pterostichus oblongopunctatus (Fabricius, 1787)	1		8	1
	Trichotichnus laevicolis (Fabricius, 1787)	2		4	
Diptera					
Athericidae	*Atherix ibis (Fabricius, 1798)*			2	
	Ibisia marginata (Fabricius, 1781)			6	
Dixidae	*Dixella aestivalis (Meigen, 1818)*	14	9		
Empididae	*Dolichocephala irrorata (Fallén, 1816)*			4	
	Dolichocephala oblongogutata (Dale 1878)			1	
	Chelifera aperticauda (Collin, 1927)			1	
	Wiedemannia braueri (Mik, 1880)				6
Limoniidae	*Arctoconopa melampodia (Loew, 1873)*				20
	Dactylolabis (Dactylolabis) transversa (Meigen, 1804)			7	
	Dicranomyia (Melanolimonia) caledonica (Edwards, 1926)			6	
	Dicranomyia (Melanolimonia) occidua (Edwards, 1926)			2	
	Dicranomyia conchifera (Strobl, 1901)		1		
	Dicranomyia mitis (Meigen, 1830)			4	
	Dicranomyia ornata (Meigen, 1818)				1
	Dicranota modesta (Osten Sacken, 1869)		1		2
	Epiphragma ocellare (Linnaeus, 1761)			1	
	Epiphragma ocellare (Linnaeus, 1761)				2
	Erioptera flava (Brunetti, 1912)	19	7		
	Erioptera griseipennis (Meigen, 1838)				2
	Helius (Helius) longirostris (Meigen, 1818)	3			
	Hexatoma (Hexatoma) bicolor (Meigen, 1818)				4
	Hexatoma (Hexatoma) fuscipennis (Curtis, 1836)				15
	Hoplolabis (Parilisia) areolata (Siebke, 1872)				10
	Limonia macrostigma (Schummel, 1829)			2	
	Limonia nigropunctata (Schummel, 1829)				1
	Limonia nubeculosa (Meigen, 1804)			3	
	Limonia phragmitidis (Schrank, 1781)				2
	Metalimnobia quadrimaculata (Linnaeus, 1761)	1			
	Molophilus ater (Meigen, 1804)	23	22		
	Molophilus pseudopropinquus (Mendl, 1973)				5
	Ormosia lineata (Meigen, 1804)			1	
	Phylidorea ferruginea (Meigen, 1818)	12	12		
	Pseudolimnophila (Pseudolimnophila) lucorum (Meigen, 1818)	4			1
	Rhabdomastix subparva (Starý, 1971)				
	Symplecta hybrida (Meigen, 1804)				3

	Tasiocera (Dasymolophilus) exigua (Savchenko, 1973)			5	
Pediciidae	*Dicranota fuscipennis* (Lackschewitz, 1940)			1	
	Tricyphona immaculata (Meigen, 1804)	1	3	1	2
Ptychopteridae	*Ptychoptera scutellaris* (Meigen, 1818)	2			
Syrphidae	*Anasimyia lineata* (Fabricius, 1787)	2	3		
	Meliscaeva auricollis (Meigen, 1822)	2	2		
	Parhelophilus versicolor (Fabricius, 1794)	1			
	Platycheirus granditarsus (Förster, 1771)	1			
	Rhingia rostrata (Linnaeus, 1758)			2	
	Sphaerophoria philantha (Meigen, 1822)	1			
Tipulidae	*Dolichopeza albipes* (Ström, 1768)			1	
	Nephrotoma appendiculata (Pierre, 1919)			2	
	Tipula luna (Westhoff, 1879)		2		
	Tipula maxima (Poda, 1761)			2	
Ephemeroptera					
Leptophlebiidae	*Habroleptoides confusa* (Sartori & Jacob, 1986)			14	11
	Habrophlebia lauta (Eaton, 1884)			8	
	Paraleptophlebia (Lestage, 1917) sp.			5	
Caenidae	*Caenis* (Stephens, 1835) sp.				1
	Caenis luctuosa (Burmeister, 1839)				3
	Caenis robusta (Eaton, 1885)				3
	Caenis horaria (Linnaeus, 1758)				4
Ephemerelidae	*Ephemerella mucronata* (Bengtsson, 1909)			1	
	Ephemerella ignita (Poda, 1796)				52
	Torleya major (Klapánek, 1905)			1	
Ephemeridae	*Ephemera danica* (Muller, 1764)			12	3
Baetidae	*Baetis alpinus* (Pictet, 1843-1845)			3	
	Baetis fuscatus/scambus			1	8
	Baetis lutheri (Muller-Liebenau, 1967)			412	
	Baetis rhodani (Pictet, 1843-1845)			91	38
	Baetis vardarensis (Ikonomov, 1962)				1
	Baetis vernus (Curtis, 1834)				23
	Baetis muticus (Linnaeus, 1758)			145	8
	Baetis niger (Linnaeus, 1761)			2	
	Centroptilum luteolum (Muller, 1776)			38	
	Cloeon dipterum (Linnaeus, 1761)	101	78		30
Heptageniidae	*Ecdyonurus insignis* (Eaton, 1870)				11
	Ecdyonurus macani (Thomas & Sowa, 1970)				2
	Ecdyonurus torrentis (Kimmins, 1942)				2
	Ecdyonurus cf. stanmachi (Sowa, 1971)			7	
	Epeorus assimilis (Eaton, 1885)			1	
	Electrogena (Zurwerra & Tomka, 1985) sp.				1
	Rhithrogena semicolorata (Curtis, 1834)				29
Oligoneuridae	*Oligoneuriela rhenana* (Imhoff, 1852)				64
Plecoptera					
Nemouridae	*Protonemura auberti* (Illies, 1954)	1		7	
	Protonemura intricata (Ris, 1902)			11	4
	Nemoura cinerea (Retzius, 1783)	11			
	Nemoura marginata (Pictet, 1835)			1	
Leuctridae	*Leuctra albida* (Kempny, 1899)	1			
	Leuctra aurita (Navas, 1919)				3

	Leuctra braueri (Kempny, 1898)			1	
	Leuctra hippopus (Kempny, 1899)	4		2	
	Leuctra inermis (Kempny, 1899)			4	
Perlodidae	*Isoperla oxylepis* (Despax, 1936)				44
Perlidae	*Perla marginata* (Panzer, 1799)			5	3
Chloroperlidae	*Siphonoperla torrentium* (Pictet, 1841)				5
Trichoptera					
Rhyacophilidae	*Rhyacophila* (Pictet, 1834) sp.			9	
	Rhyacophila dorsalis (Curtis, 1834)				4
	Rhyacophila obliterata (McLachlan, 1863)			3	
	Rhyacophila tristis (Pictet, 1835)			20	
Philopotamidae	*Philopotamus montanus* (Donovan, 1813)			3	
Hydropsychidae	*Hydropsyche* (Pictet, 1834) sp.			9	
	Hydropsyche incognita (Pitsch, 1933)				14
	Hydropsyche instabilis (Curtis, 1834)			33	6
	Hydropsyche saxonica (McLachlan, 1884)			2	
Polycentropodidae	*Holocentropus* (McLachlan, 1878) sp.				1
	Polycentropus flavomaculatus (Pictet, 1834)			2	
Psychomyiidae	*Psychomyia pussila* (Fabricius, 1781)				4
	Tinodes rostocki (McLachlan 1878)			11	
Limnephilidae spp.	*Anabolia furcata* (Brauer, 1857)				2
	Anabolia brevipennis (Curtis, 1834)		5		
	Limnephilus (Leach,1815) sp.	5	5		2
	Limnephilus auricula (Curtis, 1834)	1			
	Limnephilus stigma (Curtis, 1834)	14	12		
	Chaetopteryx fusca/villosa				3
	Allogamus auricolis (Pictet, 1834)			6	
	Halesus digitatus/tesselatus			21	1
	Potamophilax luctuosus/latipennis			6	
Lepidostomatidae	*Lasiocephala basalis* (Kolenati, 1848)				6
	Lepidostoma hirtum (Fabricius, 1775)				8
Leptoceridae	*Athripsodes aterrimus* (Stephens, 1836)				3
	Athripsodes bilineatus (Linnaeus, 1758)				2
Sericostomatidae	*Sericostoma personatum* (Spencer, 1826)			2	
Odontoceridae	*Odontocerum albicorne* (Scopoli, 1763)			5	
Odonata					
Calopterygidae	*Calopteryx splendens* (Harris, 1782)				2
	Calopteryx virgo (Linnaeus, 1758)				3
Platycnemidae	*Platycnemis pennipes* (Pallas, 1771)				20
Coenagrionidae	*Ischnura elegans* (Vander Linden, 1823)				1
	Coenagrion hastulatum (Charpentier, 1825)	2	2		
	Coenagrion hastulatum (Charpentier, 1825) *(exuvium)*	5			
	Coenagrion puella (Linnaeus, 1758)	1	1		
Aeshnidae	*Aeshna cyanea* (Muller, 1764)		1		2
Gomphidae	*Onychogomphus forcipatus* (Linnaeus, 1758)				1

Appendix B

Checklist of determined flatworms, isopods, malacostracan crustaceans, water true bugs and lacewings species and number of encaptured specimens found at four SCI sites, Veľké Osturnianske jazero lake, Male Osturnianske jazerá lakes, Jarabinský prielom gorge and Plavečské štrkoviská gravel deposit, Pieniny National Park, in the early summer 2015. Abbreviations and notes: Site n.1-Veľké Osturnianske jazero lake, Site n.2-Malé Osturnianske jazerá lakes, Site n.3-Jarabinský prielom gorge, Site n.4-Plavečské štrkoviská gravel deposit.

		Site n.1	Site n.2	Site n.3	Site n.4
Turbellaria					
Dugesiidae	*Dugesia gonocephala* (Dugés, 1830)			12	
Isopoda					
Asellidae	*Asellus aquaticus* (Linnaeus, 1758)				6
Amphipoda					
Gammaridae	*Gammarus fossarum* (Koch, 1836)			126	22
Heteroptera					
Notonectidae	*Notonecta* (Linnaeus, 1758) sp.	2			3
	Notonecta glauca (Linnaeus, 1758)	2	1		
	Notonecta viridis (Delcourt, 1909)				1
Corixidae	*Hesperocorixa* (Kirkaldy, 1908) sp.	6			
	Hesperocorixa linnaei (Fieber, 1848)	2			
	Hesperocorixa sahlbergi (Fieber, 1848)	1	1		
	Sigara (Fabricius, 1775) sp.				1
	Sigara nigrolineata (Fieber, 1848)				1
Gerridae	*Gerris* (Fabricius, 1794) sp.	4			5
	Gerris lacustris (Linnaeus, 1758)	8	3		3
Neuroptera					
Sialidae	*Sialis lutaria* (Linnaeus, 1758)		2		
Sisyridae	*Sisyra sp.*	1			

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Hematological indices of environmental pollution in the snow vole (*Chionomys nivalis*) population, High Tatra Mountains, the Western Carpathians

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Abstract. This study was focused on the monitoring of hematological parameters in the population of the snow vole *Chionomys nivalis* with regard to heavy metals pollution of the environment. We also tried to find differences in blood parameters of individuals based on age and sex. Samples were taken at Dolina Bielych plies, during sommer and autumn 2016. Statistical analysis was created for 14 blood parameters. No correlation was found between hematological parameters or age\gender and heavy metals.

Key words: *Chionomys nivalis*, hematology, hematological parameters, pollution

Introduction

One of the biggest challenges of mankind in the 21st century is that of environmental pollution caused by anthropogenic activity (Martin and Griswold 2009, Govind and Madhuri 2014). Heavy metals pose a significant hazard to humans, animals, and the health of ecosystems (Long *et al.* 2002). Alpine habitats and mountains in general work as a natural barrier for clouds and atmospheric flow and hence are particularly prone to deposition of atmospheric pollutants due to considerably higher amounts of precipitation (White 1949, Lovett and Kinsman 1990).

Blood indices are important for studying adaptive mechanisms in animals (Kostelecka-Myrcha 1967). Blood samples are also a suitable marker to be used for monitoring past pollution events (Roscales *et al.* 2010, Maceda-Veiga *et al.* 2015).

Various studies have used small mammals as bio-indicators (Martin and Coughtry 1982, Wern 1986, Talmage and Walton 1991). Snow voles are often used as a bio-monitoring species because of their specific reactions, including substantial increases in the percentage of chromosome aberrations, and changes in hematological indices. They also have fairly short life span and good reproduction rates (Topashka-Ancheva *et al.* 2003). It may be beneficial to continue studying the influence of unfavorable aspects of the environment in the context of anthropogenic pollution.

Physiological and taxonomic studies of small mammals are more and more often using hematological characteristics, which serve as an important tool for these studies. Damaged areas, where the quality of life is reduced, may cause physiological stress in mice (Pérez-Suárez *et al.* 1990). With the help of hematological data it is possible to identify conditions of individuals and populations of animals in nature that are affected by pollutants or suffer from diseases (Rostal *et al.* 2012). Changes in hematology can be caused by a variety of factors including the breed, gender, age, reproductive status, seasonal variations, and environmental parameters in areas with occurrence of pollution. Biochemical parameters of plasma are also good for determination of diseases or infection. Their levels are based on the general physiology of the organism (Gorriz *et al.* 1996). Counts of erythrocytes, as well as concentrations of hematocrit and hemoglobin indicate the oxygen transport capacity of the blood (Tersago *et al.* 2004), while reduction in the number of leucocytes can lead to decreased immunity, resulting in susceptibility of individuals to disease (Rogival *et al.* 2007).

The main goal of this study was taking blood samples from the orbital sinus of trapped snow voles to determine values of blood parameters for each sample of *Chionomys nivalis*. Following this task we began to investigate the mutual relationship between individual hematologic parameters, sex, age, and data on accumulated elements in tail vertebrae.

Material and Methods

The samples were collected in the High Tatras, Dolina Bielych Plies (valley) (see Fig. 1), in the moraine under the southern wall of Jahňací peak. In this valley there are several small shallow tarns. The largest of these is the Veľké Biele pleso, which lies at an altitude of 1613 m above sea level.

Animals were captured using Sherman live traps. Traps were baited with fresh apple to provide water supply, oat flakes and peanut butter as a main bait, and straw or sedge to support thermoregulation. At each sample site about 90 traps were set up at dusk, checked at dawn and then reset for day-trapping with a check during late afternoon. The traps were set up every 5 meters, covering

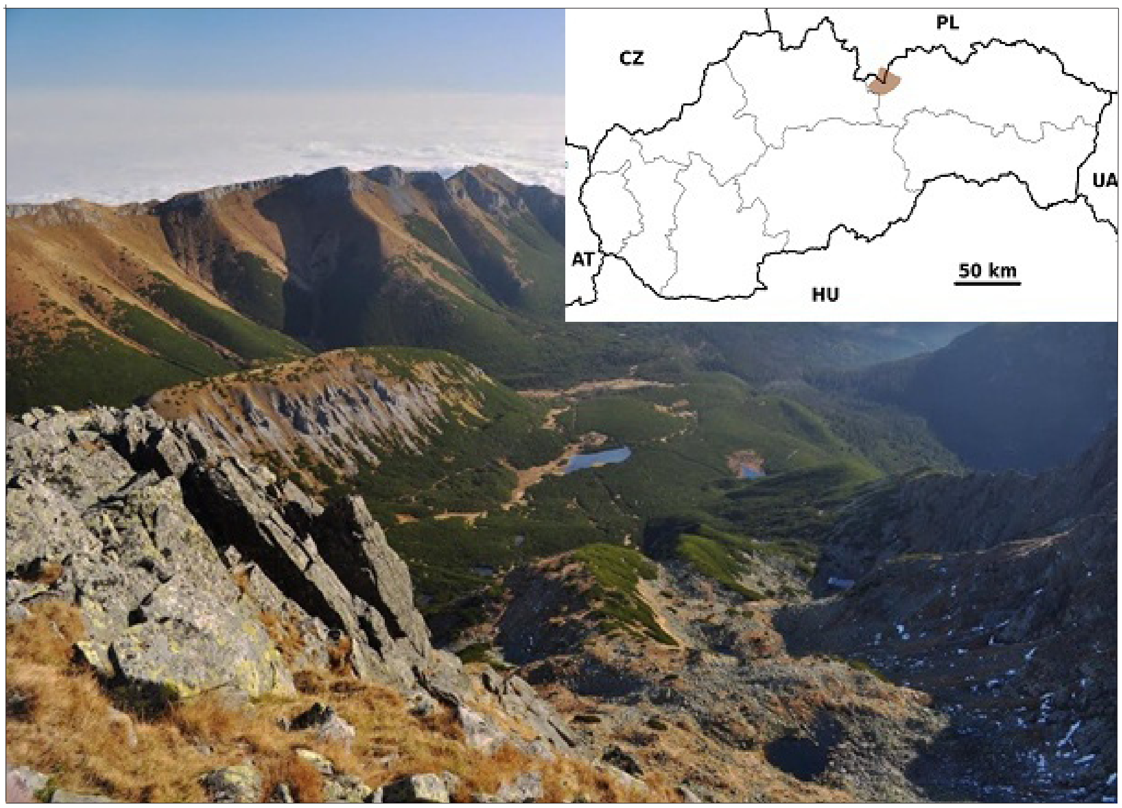

Fig. 1. Valley of white tarns and position in Europe (www.panoramio.com Igor Marhevsky, sk.wikipedia.org).

multiple types of potential habitat, including rock, dwarf pine, and small islands of grass.

Live animals were weighed in the field with a spring scale, and measurements of the following body parameters were taken: body length (without tail), tail, hind foot, and ear lobe. These morphological measurements provided information on the age category of the animal. Two different categories were defined: adults and first years (juveniles and sub-adults). The animals were sexed based on the distance between anus and papilla. Blood was taken using a capillary to access the orbital sinus, and blood was immediately transferred to tubes treated with heparin. Bleeding was stopped as soon as possible with a piece of gauze. The residual blood in the capillary was used to make blood smears. A piece of tail about 3 mm long was also taken. Following collection of this sample, the bleeding was stopped again using the method mentioned above. Collected material was placed in a portable freezer and transported to the laboratory for further analysis.

All blood samples were analyzed immediately after returning to the laboratory (no more than four hours from sampling) with the BC-2800Vet Auto Hematology Analyzer (Shenzhen Mindray Bio-medical Electronics Co., Ltd, China). The following parameters were measured: white blood cells (WBC), lymphocytes (Lymph), motocytes (Mon), granulocytes (Gran), Lymph%, Mon%, Gran%, red blood cells (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hematocrit (HCT), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and 3 histograms were made: WBC histogram, RBC histogram and PLT histogram.

Statistics were calculated in Statistica ver. 12 (StatSoft) using means, medians, minimum and maximum values, and standard deviations.

As a part of the descriptive statistics the distribution of data was tested with the Shapiro Wilk W test in Statistica software, wherein the null hypothesis is that data are from normally distributed populations. This was tested to see if the following statistics may be performed with parametric tests. After the test of normality, an analysis of variance by rank was used to investigate the difference in measured variables between two categories: sex and age class.

Advanced statistics included principal component analysis (PCA). Two of these analyses were performed: firstly, to observe relationships within hematological parameters and secondly, to investigate the possible relationship between hematological parameters and accumulation of elements in sample tails.

Results

We successfully trapped 45 snow voles in total, including 12 retraps. From this sample size we were able to collect and analyze blood in 38 individuals. The balance of sampled voles did not provide enough blood for testing. In 12 cases the analyzer could not retrieve data for all measured variables, and this was due to a low amount of sample material in the collection tube. These samples were expelled from further analyses. The following analysis thus presents results from only 26 cases and therefore must be evaluated with great caution.

The results from descriptive analysis are presented in Table 1. There were no significant dif-

		Valid N	Mean	Median	Min	Max	SD	Ref. range
WBC	(x 10^9/L)	26	320.5	346.1	98	423.5	76.5	3.2 - 12.7
Lymph #	(x 10^9/L)	26	181.2	175.3	21.5	335.2	107.3	0.6 - 5.7
Mon #	(x 10^9/L)	26	15	12.8	2.4	40.1	8.9	0.0 - 0.3
Gran #	(x 10^9/L)	26	124	113	9.9	285.8	94.9	0.2 - 1.2
Lymph	%	26	57.1	63	8.3	90.3	30.9	60 - 95
Mon	%	26	4.4	4	1.9	10.6	2.1	3.5 - 5.0
Gran	%	26	38.5	31.4	7.8	88.4	29.9	8.6 - 38.9
RBC	(x 10^{12}/L)	26	2	1.5	0.2	6.8	1.8	7.0 - 10.1
HGB	(g/L)	26	78.5	44.3	3	319	81.7	118 - 149
HCT	(%)	26	7.9	6.2	1	24.4	6.5	36.7 - 46.8
MCV	(fL)	26	43.4	43.6	33.1	61.4	7.5	42.2 - 59.2
MCH	(pg)	26	37	41.3	12.8	66.6	13.2	13.8 - 18.4
MCHC	(g/L)	26	921.3	1035	272	1413	403.2	302 - 353
RDW	(%)	26	22.3	21.2	12.5	35.5	7.6	13.0 - 17.0
PLT	(x 10^9/L)	26	97.6	91	22	262.5	53.8	766 - 1657
MPV	(fL)	26	7.3	6	5.4	34	5.5	6.0 - 6.5
PDW		26	14.9	14.9	14.4	17.1	0.6	15.7 - 16.3
PCT	(%)	26	0.1	0.1	0	0.3	0.1	0.049 - 0.128

Table 1. Description of measured variables N: number of valid cases; Min: minimal value; Max: maximal value; SD: standard deviation; Ref.range - reference range for laboratory mouse drawn from hematological analyser. Variables in bold comes from Normal distribution – Shapiro-Wilk W test.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
WBC	-0.242	-0.453	0.051	**0.758**	0.308	0.119
Lymph # H	**-0.839**	-0.030	-0.394	0.336	0.055	0.017
Mon #	0.269	**-0.874**	0.173	0.318	0.010	0.126
Gran #	**0.833**	-0.169	0.496	0.048	0.127	0.045
Lymph %	**-0.898**	0.037	-0.427	0.026	-0.057	0.021
Mon %	0.423	**-0.852**	0.144	0.174	-0.063	0.105
Gran %	**0.895**	0.022	0.431	-0.041	0.062	-0.029
RBC	**0.927**	0.313	0.003	0.021	0.093	-0.065
HGB	**0.939**	0.259	-0.110	-0.001	0.024	-0.049
HCT	**0.893**	0.352	0.191	0.035	0.084	-0.052
MCV	-0.559	0.215	**0.757**	0.126	-0.064	-0.018
MCH	0.702	-0.342	**-0.489**	-0.042	-0.162	0.020
MCHC	0.695	-0.323	**-0.595**	-0.129	-0.058	0.049
RDW	-0.675	0.346	0.220	0.450	-0.072	-0.227
PLT	0.601	0.480	-0.131	0.501	-0.341	0.104
MPV	0.028	**-0.705**	0.129	0.035	-0.459	-0.500
PDW	-0.510	-0.118	0.420	-0.372	-0.467	0.387
PCT	0.514	0.499	-0.117	0.540	-0.393	0.122
% Variation	47.1	18.8	12.8	9.7	4.9	3.0

Table 2. PCA analysis of hematological parametres. Results of interest are presented in bold.

ferences in the distribution of any of the measured parameters due to sex or age class.

The analysis performed was the PCA of hematological parameters (see Table. 2). The most important factors were Factor 1, 2 and 3. Factor 1 denotes an inverse relationship between the lymphocytes and Gran, HGB, RBC and HCT.

Factor 2 suggested a positive relationship be-

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
WBC	0.222	0.173	0.482	0.164	**0.756**	-0.203	-0.064	-0.050
Lymph # H	**0.728**	0.477	0.046	-0.312	0.353	-0.008	-0.002	0.063
Mon #	-0.176	-0.222	**0.862**	0.220	0.282	0.021	0.132	-0.051
Gran #	**-0.721**	-0.444	0.170	0.465	0.030	-0.125	-0.048	-0.098
Lymph %	**0.801**	0.423	-0.046	-0.400	0.049	0.064	0.061	0.036
Mon %	-0.307	-0.316	0.835	0.167	0.140	0.079	0.143	-0.025
Gran %	**-0.803**	-0.413	-0.011	0.401	-0.062	-0.070	-0.072	-0.036
RBC	**-0.918**	-0.190	-0.249	0.028	0.023	-0.035	-0.180	0.066
HGB	**-0.941**	-0.164	-0.194	-0.076	-0.003	0.033	-0.119	0.066
HCT	**-0.871**	-0.227	-0.300	0.202	0.027	-0.078	-0.162	0.072
MCV	**0.561**	0.052	-0.281	**0.734**	0.052	-0.033	0.089	-0.045
MCH	**-0.656**	-0.213	0.381	-0.480	-0.001	0.106	0.115	0.076
MCHC	**-0.666**	-0.164	0.374	-0.587	-0.072	0.054	0.039	0.036
RDW	**0.649**	0.128	-0.393	0.218	0.444	0.080	0.060	-0.002
PLT	**-0.644**	-0.023	-0.435	-0.042	0.463	0.331	0.159	0.133
MPV	0.016	-0.022	**0.698**	0.221	-0.088	0.413	0.099	0.278
PDW	**0.528**	-0.003	0.019	0.348	-0.435	0.059	0.501	0.140
PCT	**-0.561**	0.006	-0.460	-0.022	0.492	0.389	0.180	0.130
S	-0.173	**0.882**	0.154	0.229	-0.047	-0.001	-0.180	0.095
Cl	**-0.536**	0.669	0.043	0.033	0.112	-0.398	0.093	0.057
K	**-0.533**	**0.720**	0.157	-0.047	-0.009	-0.294	0.125	-0.059
Ca	-0.042	**0.799**	0.268	0.129	-0.098	0.285	-0.202	-0.167
Cr	-0.376	**0.795**	-0.092	0.092	-0.086	0.339	-0.034	0.117
Mn	-0.244	**0.864**	0.174	-0.079	-0.089	-0.175	-0.021	-0.064
Zn	-0.401	**0.836**	0.076	0.110	0.021	-0.078	0.111	0.126
Rb	**-0.653**	0.422	-0.270	0.053	-0.155	-0.060	0.392	0.137
Mo	-0.394	0.203	-0.087	-0.032	-0.054	**0.561**	0.174	**-0.622**
Ba	-0.015	**0.791**	0.119	0.210	-0.102	0.352	**-0.332**	0.102
Pb	**-0.474**	0.568	-0.153	-0.052	0.095	-0.341	0.222	-0.254
% Variation	32.4	23.4	12.4	7.8	6.0	5.4	3.2	2.5

Table 3. Principal component analysis PCA of hematological parameters and concentrations of elements accumulated in tail vertebrae of snow vole.

tween monocytes and MPV. Factor 3 described an increased relationship between MCV and MCH MCHC.

The second PCA investigated the relationship between hematological parameters and accumulation of elements in tails, vertebrae and soft tissue. These results are presented in Table 3. Most of the variation was again explained by the same three factors as above.

The results show that hematological indices have their own variability (Factor 1, Factor 3) which is independent of variability of elements in bones (Factor 2).

Discussion

Statistical analysis was performed after gathering all necessary data into a large dataset. The first analytical method used was Kruskal-Wallis non-parametric version of ANOVA. In this test, we tried to compare blood parameters to gender and age. This test attempts to detect differences among the population means (Kruskal and Wallis 1952). No significant differences were found in males versus females or between adults and sub-adults. However in a study by Wołk and Kozłowski (1989), who studied the population density of *Apodemus flavicollis* along with hematological parameters, there were observed changes in RBC and WBC connected to sex and age of the animals. In the study of Beldomenico *et al.* (2008) changes were observed in hematological parameters in the wild field vole population connected to sex and age.

Two PCA analyses were also performed, the first one was done to see the relationship between the measured parameters. The most important factors were factor 1, factor 2 and factor 3. Factor 1 is an inverse vector describing an inverse trend between increased lymphocyte parameters and granulocytes, RBC, HCB and HTC or vice versa.

Leucocyte profiles are altered by stress and can be directly related to stress hormone levels. Stress or treatment by glucocorticoid can cause changes in a number of leucocytes. Glucocorticoids act to increase the number and percentage of neutrophils, while decreasing the number and percentage of lymphocytes. This is a phenomenon we see in all vertebrates in response to natural stressors. High numbers of heterophils or neutrophils to lymphocytes indicate high levels of glucocorticoids (Davis *et al.* 2008), while the numbers of lymphocytes decrease during immunosuppressive infections (Beldomenico *et al.* 2008). The most important factors affecting the erytrocyte count - hemoglobin and hematocrit - change based on the test environment's temperature and photoperiod. In different species of rodents, erythrocyte, hemoglobin and hematocryt had the highest values during the winter (Rewkiewiccz-Dziarska 1975). This is partly because animals exhibit higher metabolic rates in order to acclimatize to lower winter temperatures (Pérez-Suárez *et al.* 1990). Lee and Brown (1970), who studied burrowing rodents, found that during winter when there can be a lack of oxygen, the response is an increase in HGB concentration. The hematocrit value is dependent on changes in the number and size of the erythrocytes (Kostelecka-Myrcha 1967).

The second factor is a unipolar vector describing mutual decrease or increase of monocytes and MPV. Monocytes are associated with defence against infections and bacteria (Campbell 1995, Davis *et al.* 2004). Low monocyte levels might suggest insufficient immunocompetence to mount an inflammatory response (Beldomenico *et al.* 2008). In patients with large numbers of megacaryocytes, (megacaryocytic hypoplasia) decreased MPV is recorded (Wintrobe 2009). The third factor suggested an inverse relationship between MCV and MCH/MCHC. MCV is useful in classification of anemia. In a study by Pérez-Suárez *et al.* (1990), seasonal variation in MCHC in *Pitymys duodecimcostatus* was observed. This index reflects erythropoeisis, and can be affected by diet-defieciencies of iron or lack of oxygen. Çetin *et al.* (2009) observed lower erythrocyte counts and hemoglobin concentrations in pregnant rabbits compared with non-pregnant rabbits, while mean corpuscular volume was higher in pregnant rabbits than in non-pregnant rabbits. They also found changes in total leucocyte counts and lymphocyte ratios which were lower in pregnant females. Due to gravidity, some hematological parameters may decrease (Doubek *et al.* 2003).

In the second PCA we did not find any significant mutual variation between hematological parameters and amounts of measured elements in the tail vertebrae of voles. In a number of studies (Gorriz *et al.* 1996, Rogival *et al.* 2006, Tete *et al.* 2015, Waghmare *et al.* 2015), it was observed that environmental pollution had a negative impact on hematological parameters of living organisms. This may cause different diseases or anemia. Lymphopenia was reported, as well as neutrophilia, in most in studies where fish were exposed to heavy metals (lead, zinc, copper, cadmium) (Davis *et al.* 2008). Nunes *et al.* 2001 found higher concentrations of mean corpuscular hemoglobin in the Algerian mouse (*Mus spretus*) in polluted areas compared to the reference site. The results of a study by Rogival *et al.* (2006) indicate that metal exposure can have a negative impact on the oxygen-transport capacity of blood. Early warning signals of this include decreased hematocrit levels.

In this study we have successfully measured hematological parameters of trapped snow voles, creating a reference point for future research in ongoing projects on bio-indication of *Chionomys nivalis*. We also fulfilled the ancilliary goal of further investigation of this data; however this was only partial successful as the sample size was not large enough to draw any firm conclussions.

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

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

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

Introduction

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

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

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

Material and Methods

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

Live traps were distributed in the field approximately 5 to 10 m apart from one another. Approximately 80 traps were checked per day. The fieldwork took place over 18 days. Coordinates were recorded with GPS to mark the location of trapped individuals. Photo and video samples were taken for use in additional evaluation of plants, mosses, and geological features of the study area.

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

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

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

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

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

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

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

Results

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

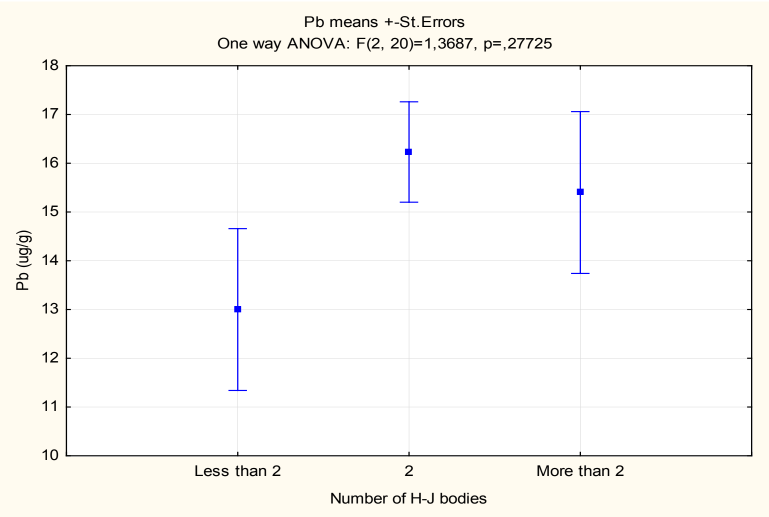

Fig. 1. Amount of lead in bones of *Chionomys nivalis* and the number of Howell-Jolly bodies in their peripheral blood.

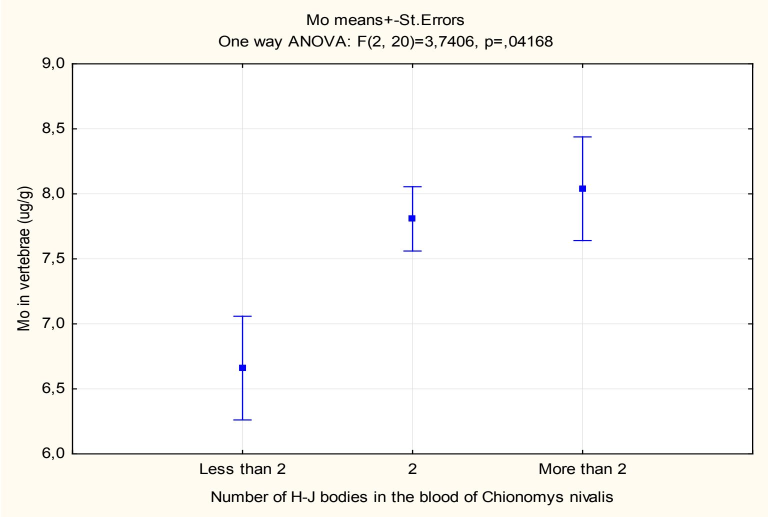

Fig. 2. Amount of molybdenum in bones of *Chionomys nivalis* and the number of Howell-Jolly bodies in their peripheral blood.

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

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

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

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

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

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

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

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

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

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Ecotoxicological assessment of *Juncus trifidus* in the Dolina Bielej vody Valley, High Tatras

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Abstract. This work focuses on detection of toxic elements in *Juncus trifidus*. The research took place in the Dolina Bielej vody Valley, in the eastern part of the High Tatras. Sampling was carried out in July, August and September. During collection the plant was divided into four parts, each to be analysed separately: the upper green part (leaves, blade, flower or spike); sheath (part of plant at the ground level); roots; and soil. Collected and dried samples were then processed and analysed in the laboratory. The samples were ground in a hand mill and then put into the roentgen which identified specific toxic elements in particular parts of the plant. This research was partially related to that on the snow vole (*Chionomys nivalis*). If toxic elements are detected in the snow vole, it is probable that this toxicity is caused in part by its diet, including *Juncus trifdus*. We observed important differences between processes of accumulation of individual elements in plant tissues. Calcium levels were highest in the green parts of plants in their germinative tissue (likely due to production of seeds). Similarly, zinc and manganese were found in the samples, as calcium, manganese and zinc are biogenic elements. We found variation in the amount of lead in soil versus in the green parts of the plant. This shows that *Juncus trifidus* doesn't accumulate this element in the tissues in high amounts.

Key words: Juncus trifidus, toxic elements, heavy metals

Introduction

Today, pollution of the environment - especially through anthropogenic activity - has a greater impact than ever before. In today´ s society there is a common misconception that natural environments that appear untouched by humans - such as the Alpine - are largely protected from these unfavourable impacts. However, environmentalists have discovered that these zones are actually some of the least protected from environmental impacts as evidenced by the relatively high amount of lead in Alpine ecosystems (Šoltés *et al.* 1992). High altitude works as a natural barrier, retaining precipitation and transmissions of various natures. As such, the altitude is a deciding factor influencing the amount of polluting substances found in an alpine environment. Thus, we decided to study the concentration of toxic elements in *Juncus trifidus*, which is one of the dominant species in the high alpine, (Zeidler and Banaš 2013) and is found in almost all high mountains in Europe.

As *Juncus trifidus* is in abundance throughout the Tatras, it was necessary to concentrate on a sample area; Dolina Bielej vody Valley in the eastern High Tatras was chosen. Additionally, the snow vole (*Chionomys nivalis*) can be found in Dolina Bielej vody Valley, and our research is bound to this rodent as *Juncus trifidus* constitutes part of its diet. Our aim is to find out whether toxic substanceds found in this animal come from the food it feeds on. The aim of this work is to prove or disprove the presence of specific polluting substances or elements in alpine environments by means of sampling and analysing *Juncus trifidus.* The main goals were:

- Identification of possible appearance of heavy metals and other toxic substances in tissues of the plant. Our goal is to determine where the concentration of these substances is highest, as well as how it is transferred from one part of the plant to another.
- Developing an explanation for deposition processes of toxic elements in plant tissues from the surroundings.
- Explain potential relationship between the content of toxic elements in *Juncus trifidus* and content of identical elements in the body of snow vole.

Societies of strongly blown (so called deflationary) slopes and peaks of alpine degree (in accelerating peak part of anemo-orographic systems (A-O systems). They are low vegetation (ca. up to 20 cm) of so called alpine grasses with *Juncus trifidus* and *Festuca supina*, which according to the relief shape and wind force, are unconnected patches with discontinuous areas of bare soil and screes with bryophytes and lichens or bare rocky bedrock (strongly exposed peak parts) to compact vegetation (less exposed slopes and areas with longer lasting snow cover). Nature and development of societies of windward positions is determined by several limit-ing factors. Firstly, there is vegetation exposed to strong disturbance from the abiotic factors, mainly wind. As a result of constantly strong air flow, there is insufficient deposition of snow cover in deflationary peak plains, ridges and peaks of alpine degree (there is often only weak ice crust in winter season). Due to insufficient insulation of snow layer there occurs intensive pergelation of upper layer of soil profile in these conditions. Therefore the snow melts early and in short time horizon in spring resulting in moisture deficit in case of rainfall shortage in this season (Zeidler and Banaš 2013).

Material and Methods

The research took place in Dolina bielej vody Valley situated in the eastern High Tatras, Slovakia. It is a valley of unusual glacial cirque type, lying at an altitude of 1600 metres, with an area of 1,8 km^2. Biely potok brook flows through it.

Work in the field consists of *Juncus trifidus* sampling. We set 10 sampling points placed evenly to occupy as much area as possible (Point 1: N: 49° 13. 541°, E: 20° 13.329°; Point 2: N: 49° 13.538°, E: 20° 13.309°; Point 3: N: 49° 13.532°, E: 20° 13.271°; Point 4: N: 49° 13.519°, E: 20° 13.237°; Point 5: N: 49° 13.509°, E: 20° 13.201°; Point 6: N: 49° 13.496°, E: 20° 13.240°; Point 7: N: 49° 13.494°, E: 20° 13.258°; Point 8: N: 49° 13.495°, E: 20° 13.285°; Point 9: N: 49° 13.504°, E: 20° 13.285°; Point 10: N: 49° 13.506°, E: 20° 13.311°). It was necessary to dig plants out of the rocky soil as we wanted to collect and retain samples of roots and soil, which needed to be collected.

The samples were taken from July until September. Specific plants were put into separate bags together, and labelled with the sampling point and date of sampling. Later, the samples were taken out and dried. All dried plants were divided into four parts that had to be analysed separately: upper green parts (leaves, blade, flower or spike); ground level parts; roots (thoroughly cleaned); and soil. After dividing the plant, all samples were ground in the hand mill to fine dust. In order to prevent contamination and results distortion it was necessary to wash the drum and metal ball under running water after each grinding and then to thoroughly dry it. Ground samples were inserted into labelled resealable bags. There were 120 samples in total. We inserted sample into special small vessel (min. 3 mm) which was then inserted into X-ray (hand ED-XRF spectrometer DELTA with setting to mode 3 beam (80-80-80) light mode).

Results and Discussion

Our research focused on toxicity in the plant *Juncus trifidus*, and we confirmed the presence of elements that appeared in varying concentrations in the plant. On the whole, we managed to confirm the presence of nineteen elements, out of which two were highly toxic; namely lead and arsenic. Arsenic could be found in trace amounts, while lead was found in much higher amounts. Analysis confirmed the presence of elements such as chromium and chlorine, which can be a burden for the ecosystem when their values reach higher levels. Remaining elements in *Juncus trifidus* were manganese, molybdenum, strontium, rubidium, calcium, copper, barium, zirconium, titanium, potassium, antimony, zinc, iron and sulphur. However, several of these elements are naturally occurring in *Juncus trifidus*, as well as many other plants.

Sulphur (S), Calcium (Ca), Manganese (Mn), Zinc (Zn)

The highest amount of sulphur was found in soils. With regard to the plant itself, the green part contained a higher amount of sulphur than dry sheaths and roots. The sulphur cycle in the plant was more or less the same in all months, and it is likely that the contamination of aboveground parts was a little higher in September (Fig. 3).

The sulphur cycle is a biogeochemical cycle. To a certain extent, there are participating organisms that decompose or synthesize various sulphuric compounds. The complexity of the sulphur cycle varies due to the level of oxidation of sulphur. Sulphur occurs naturally in the body of organisms and makes up part of the protein structure (amino acids cysteine and methionine or metalloproteins) and coenzyme structure (Stránská 2013).

High amounts of calcium can be found in aboveground parts of plants in September when the plant is already dry. Since calcium can be found in dry sheaths only in small amounts, there is high probability that calcium gets into dry parts of plants in autumn and thus is contained mainly in seeds created by plant in the spring-summer (Fig. 4). Calcium is one of the main building elements in plants and participates in various biological processes.

Calcium is quite widespread in Earth's crust: 3.5% Ca (limestones $CaCo_3$, dolomites $CaCo_3{\cdot}MgCO_3$, magnesites $MgCO_3$, gypsums $CaSO_4{\cdot}2H_2O$). Ca^{2+} gets in water by digestion from minerals. Its solubility depends on content of CO_2 in water (Orolínová 2009).

The manganese cycle is similar to the calcium cycle, with higher content in the green parts of plants after they are dried. As the amount of manganese is low in the dry sheath, we suppose that increased amounts can be found in green part of plants (Fig. 5). Manganese is relatively evenly distributed in ecosystems. It occurs in iron ores and it gets to water mostly by digestion of dead parts of plants and by digestion of minerals. Manganese can get into the environment through anthropogenic activities, including waste waters from metallurgy and metal processing. Manganese is not distributed uniformly in soil substrata and, in addition to various nodules, is also concentrated in certain spots and veins (Kabata-Pendias and Mukherjee 2007).

Zinc amounts did not differ over the sampling period. The highest amount of zinc was found in the green parts of plants. Smaller amounts of zinc were found in the dry part, and the smallest amount in root systems. Zinc content in the dry part was similar to calcium and manganese. High amounts of Zinc were found in the seeds, while a small amount of zinc was found in the soil (Fig. 6).

Plants pull zinc from the soil in the form of microelements such as the divalent cation Zn^{2+}. Its concentration in most of the plants is between 20 and 100 ug/g (Horník 2010). A shortage of zinc

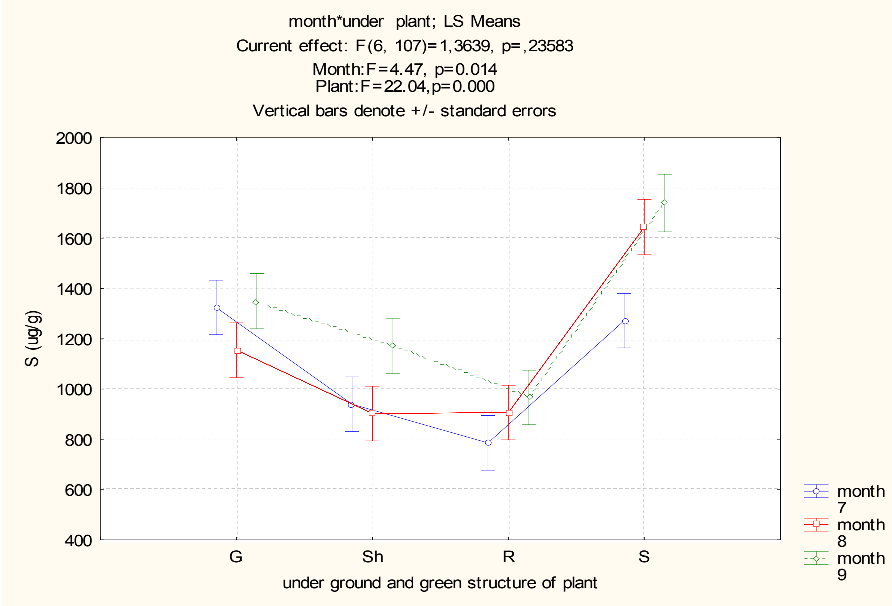

Fig. 1. The amount of sulphur was highest in the soils. The green parts of the plants contained higher levels of sulphur than the dry sheaths or roots. The cycle and distribution of sulphur was more or less equal in all months, the external contamination of habitats was significantly higher in September than in summer (July). (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

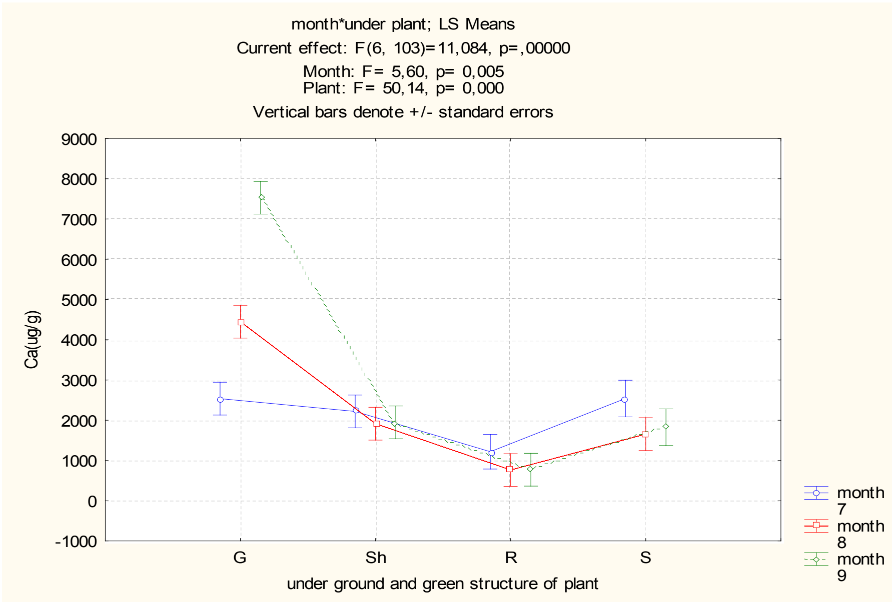

Fig. 2. The amount of calcium was highest in green parts of plants, and probably mainly in their germinative parts (seeds – September). The sheaths and roots contained less calcium than green parts. Calcium is a biogenic element. In green parts of the plant the levels of calcium were significantly higher than in the surrounding soils. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

can result in weakening of the stem and dwarfed growth (Marschner 1995).

Chlorine (Cl), Potassium (K), Rubidium (Rb)

Chlorine (Fig. 7) appears neither in water nor in the air. Chlorine is a common halogen element in terrestrial and aquatic environments (Kabata-Pendias and Mukherjee 2007). However, it can make its way into ecosystems through anthropogenic activities or unique natural processes such as volcanic activity. Sometimes chlorine enters the environment through the air by weathering of cryolite, apatite, aluminium fluoride or sodium fluoride. Chlorine is absorbed more often as a result of anthropogenic activity, such as chlorination or whitening processes or by combustion of plastic emissions.

Potassium is naturally found in feldspars and micas, which are prevalent in the soil. The underlying rock at the sample site was granite, which is composed of these two minerals for the most part (Fig. 8). These minerals are characterized by banded structure and are able to accumulate cations K^+ (Čurlík 2003). Potassium, similar to chlorine, likely binds to green parts of plants through their respiratory process.

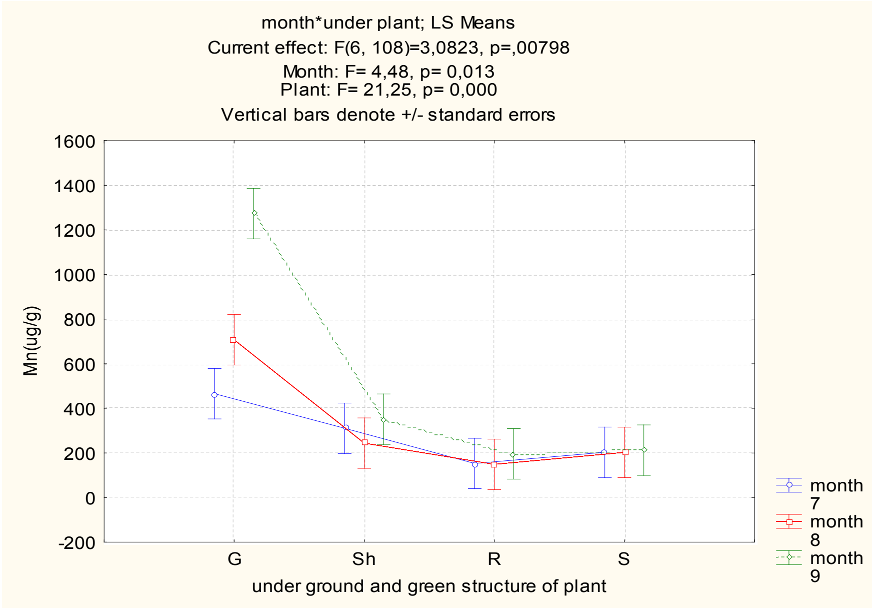

Fig. 3. Manganese cycle is similar to calcium cycle. Its amount is higher in green parts of plants after they are dried (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

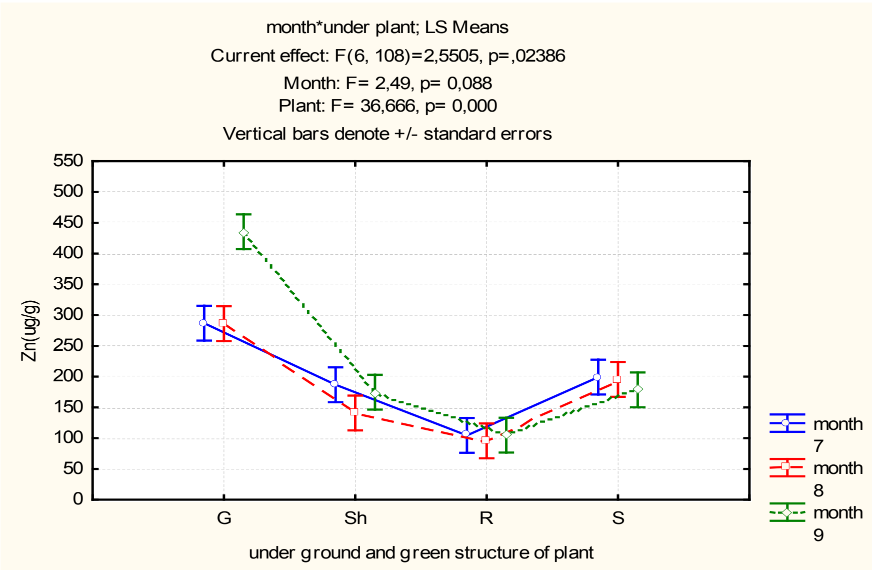

Fig. 4. Accumulation of zinc in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

Rubidium has a similar cycle to potassium and chlorine in plants (Fig. 9). It was found in much higher amounts in the soil than in the roots or dry parts. Higher concentrations of rubidium in the green parts of plants indicates that rubidium concentrates there during photosynthesis. When dried, rubidum decreases in aboveground parts and sheaths. Potassium and sodium accompany rubidium in small concentrations. Minerals containing these elements are the cause of their usual concentration increase in water. Rubidium in small concentrations forms part of the plants structure (Bencko *et al.* 1995).

Iron (Fe), Lead (Pb)

Iron (Fig. 10) is found in nature as a compound in rocks and minerals such as limonite, pyrite, magnetite, siderite or aluminosilicates. In our case, granite with a high concentration of aluminosilicates containing iron is the cause of high iron content in soil. However, iron gets in water and then in the environment only minimally by means of mineral digestion. CO_2 sulfuric acid or humic substances help iron to dissolve but it was found that the highest concentration of iron in soil occures as a result of anthropogenetic factors (Ciriaková 2009).

Lead (Fig. 11) appears in nature only as a compound in minerals such as galenite, cerusite, and anglesite. In the past, lead pollution was mainly as a result of burning fuel from transport vehicles. Today lead gets into the environment mainly from industrial activities, namely, emissions. Much lead is thus collected from the plant leafs. The smallest amount is found in fruit and seeds (Svičeková and Havránek 1993).

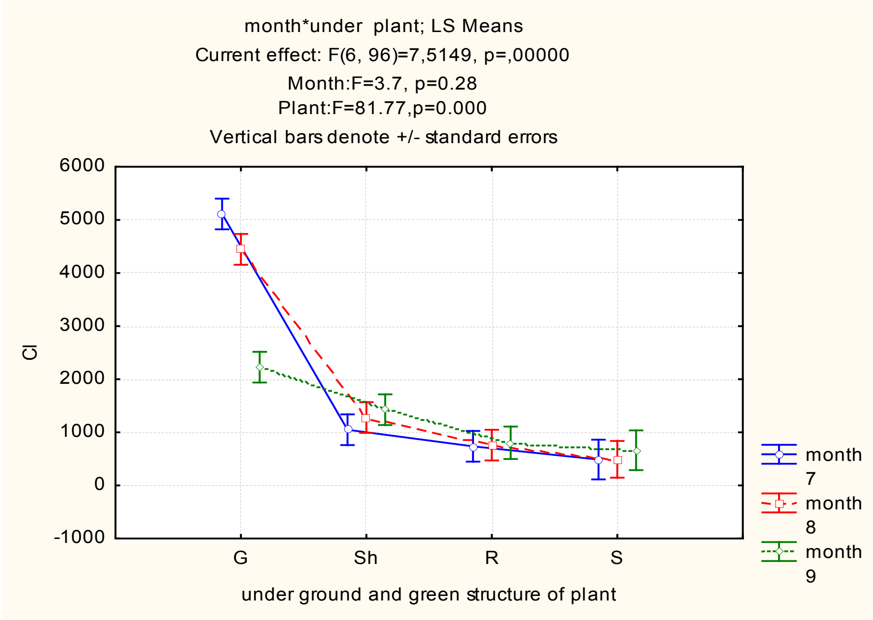

Fig. 5. The highest amount of chlorine was found on the surface of leaves and thus mainly exists in summer months (July and August). When leaves dried in September, the amount of chlorine decreased in sheaths, roots and soil, but still existed in high concentration in green parts. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

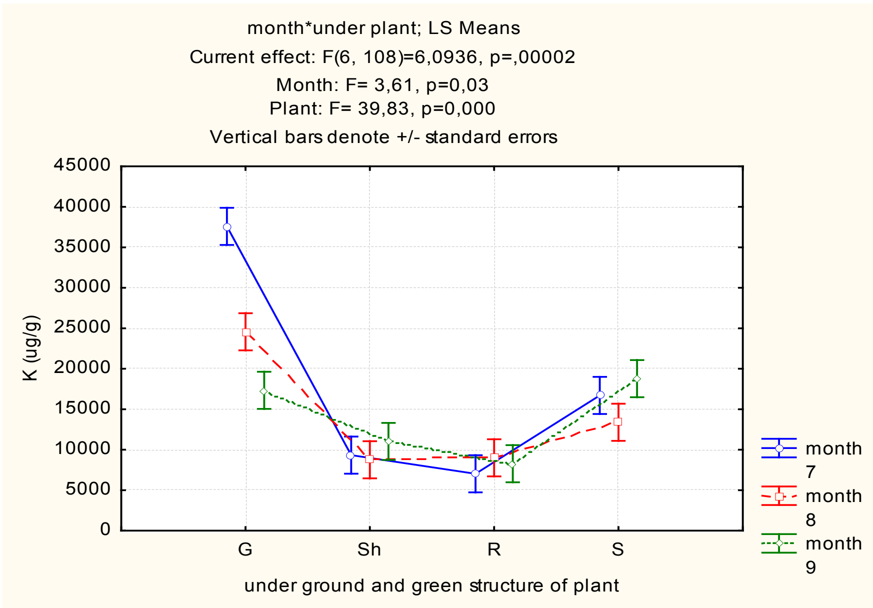

Fig. 6. Potassium, similarly to chlorine, got into green parts of plants in summer season. Potassium amounts decreased in September when plants dried. Minimum amounts were found in sheaths. There was an increased amount of potassium in soil compared to dry parts of plants. Only minimum potassium was found in roots. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

Lead has the ability to effectively bind to soil particles (Wu *et al.* 1999; Barona *et al.* 2001; Kos and Leštan 2003). The plant has limited capacity to pull lead into aboveground parts in high quantities, (Woźny 1995; Kumar *et al.* 1995; Sekhar *et al.* 2005). In our research, the highest concentration of lead was found in soils. Thus, the roots also exhibit high lead content. Our research shows a distinct difference in the amount of lead found some parts of the plant compared to others.

Chromium (Cr)

Chromium (Fig. 12) occurs in nature only as the mineral chromite. It can be released into the environment by mineral digestion, but most often gets into an ecosystem through anthropogenic activities such as industrial waste waters or from surface metal processing.

The world median content of Cr in soils has been estabilished as 54 mg kg^{-1}. Its content in soils is determined mainly by its abundance in the parent material. Since soil Cr is inherited from parent rocks, higher content is generally found in soils derived from mafic rocks and argillaceous sediments (Kabata-Pendias and Mukherjee 2007).

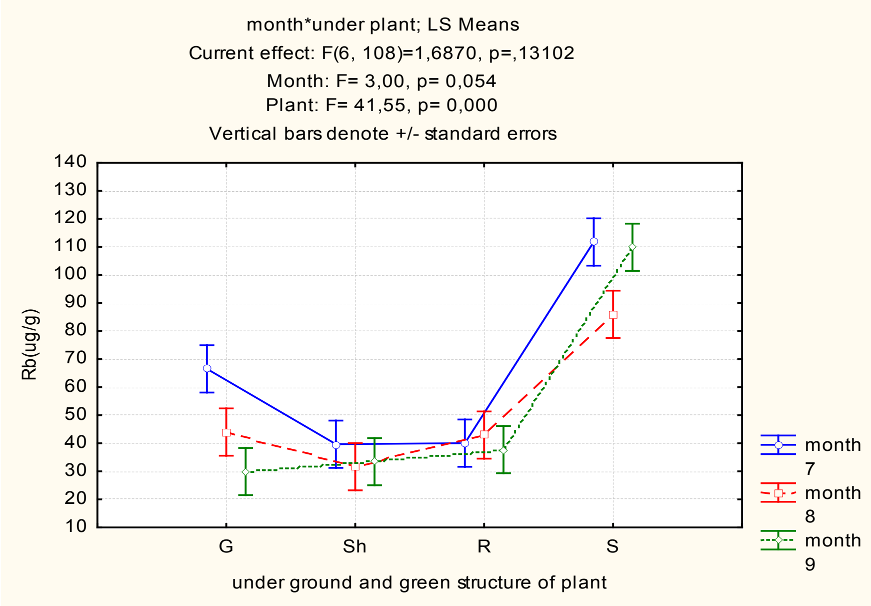

Fig. 7. Accumulation of rubidium in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

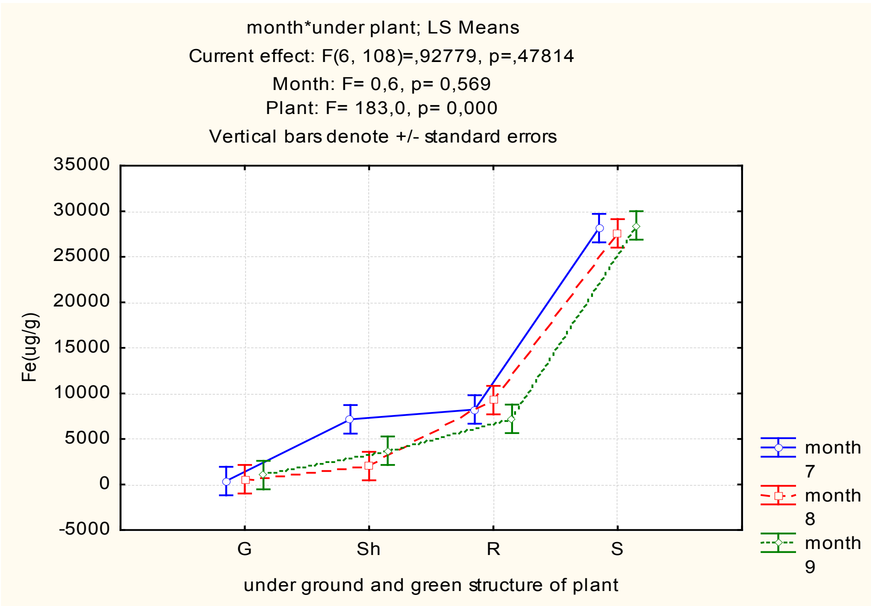

Fig. 8. Iron was found in relatively great amount in soil, but the plants had no tendency to absorb it through roots. There was a little of iron in dry parts of plants, either in summer or in autumn. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

Copper (Cu), Molybdenum (Mo), Antimony (Sb)

Small concentrations of copper get into plants though physiological processes (Fig. 13). Concentration of copper in roots and aboveground parts was similar whether they were dry or green. There a high quantity of copper in soil when the terrain was drying up in autumn.

Copper occurs in the Earth's crust at concentrations between 25-75 mg kg^{-1}. Its abundance pattern in rocks shows the tendency for the concentration in mafic igneous rocks and in argillaceous sediment, however, it is mostly excluded from the carbonated rocks (Kabata-Pendias and Mukherjee 2007).

Yong *et al.* (1992) found that optimum amounts of copper are essential to the plant. However, even essential elements necessary for the plant may become toxic if they exceed certain concentrations (Tomáš 2000).

The amount of molybdenum is relatively low in comparison with the amount found in plant bundles (Fig. 14). Molybdenum is not actively absorbed by the plant from she soil. In autumn the difference in the amounts of molybdenum in various parts of the plant increases. Molybdenum concentration is low in July, but high concentrations are found in the roots during autumn. Molybdenum is mined as a primary ore deposit, mainly as molybdenite, as well as a byproduct in copper mines. Annual produc-

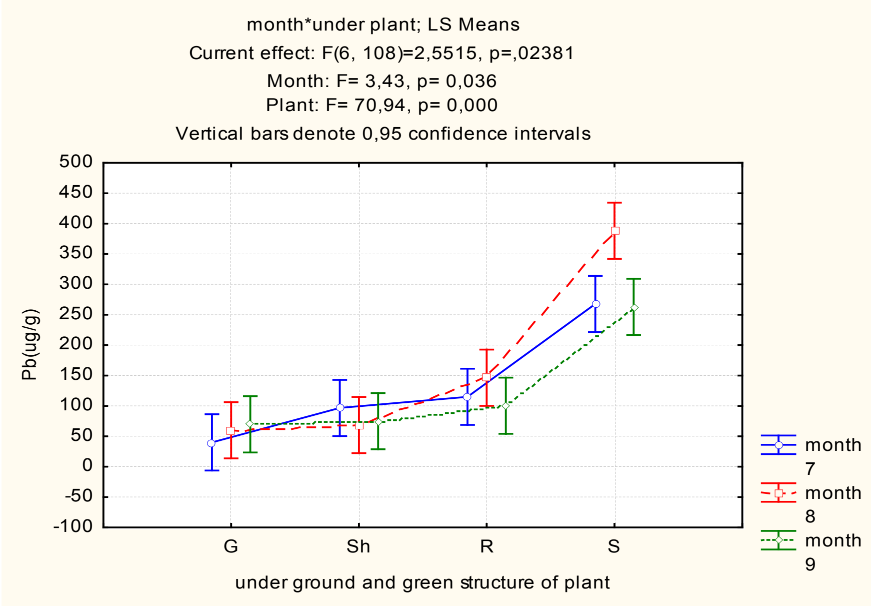

Fig. 9. Content of lead was higher in August than in July and September. Higher amount of lead was found in roots in all months in comparison to the green part of plant. Higher amount of lead in aboveground parts of plants was found in sheaths, which were already dry in July and amount of lead was high in this season. It got into roots early in the spring, and to green parts of plant and sheaths after snow melted. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

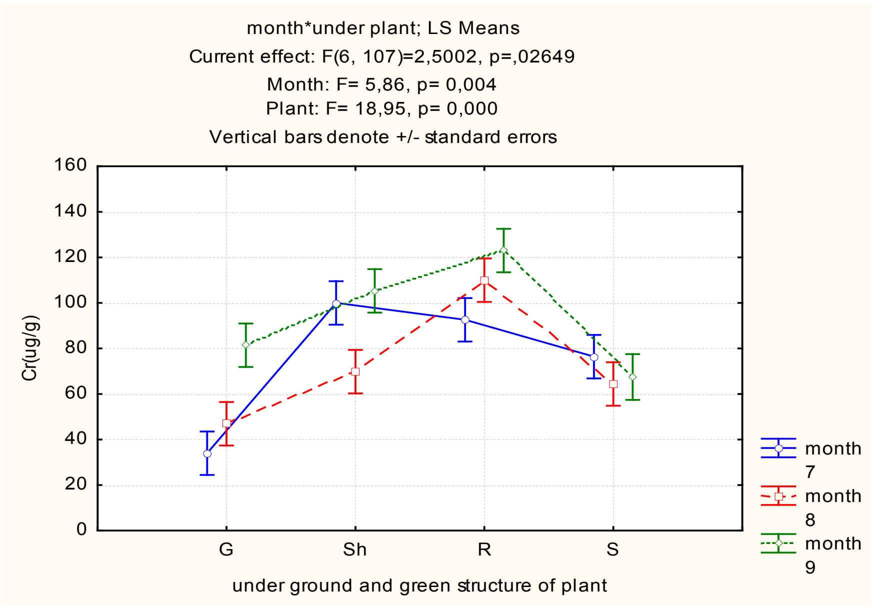

Fig. 10. Chromium amount in plant grows after all green parts have dried up in September. Chromium in sheath and green parts of plant has been balanced gradually. The highest amount is found in roots, which is much higher than in soil. The concentration in all subjects did not differ in different months. This means that plant can actively absorb chromium from soil and cumulates it only in root parts. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

tion of Mo in 2003 was 127.4 kt (WMSY 2004). Its main use is in metallurgy for the hardening of alloys (Kabata-Pendias and Mukherjee 2007).

The amount of molybdenum is relatively low in comparison with the amount found in plant bundles (Fig. 14). Molybdenum is not actively absorbed by the plant from she soil. In autumn the difference in the amounts of molybdenum in various parts of the plant increases. Molybdenum concentration is low in July, but high concentrations are found in the roots during autumn. Molybdenum is mined as a primary ore deposit, mainly as molybdenite, as well as a byproduct in copper mines. Annual produc-

tion of Mo in 2003 was 127.4 kt (WMSY 2004). Its main use is in metallurgy for the hardening of alloys (Kabata-Pendias and Mukherjee 2007).

Antimony exhibits chalcophilic properties, combines readily with sulphides and occurs mainly at 3^+ and 5^+ oxidation stages. Its content in igneous rocks ranges between 0.1 and 0.9 mg kg^{-1} and is likely to increase up to 2 mg kg-1 in argillaceous rocks (Kabata-Pendias and Mukherjee 2007).

Antimony (Fig. 15.) is absorbed by environments as an atmospheric emissio, through anthropogenic activity (mining, technologic and industrial processing), or through natural processes (forest

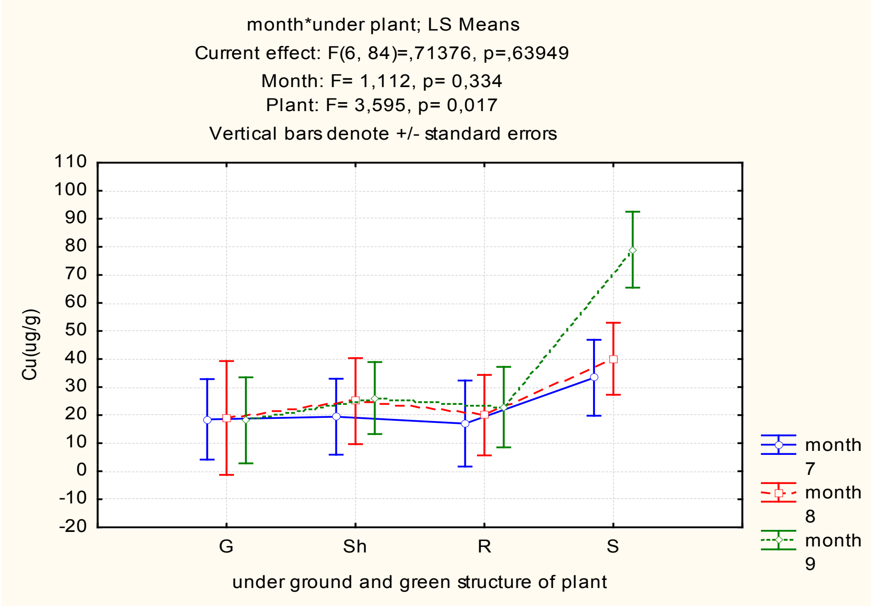

Fig. 11. Accumulation of copper in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

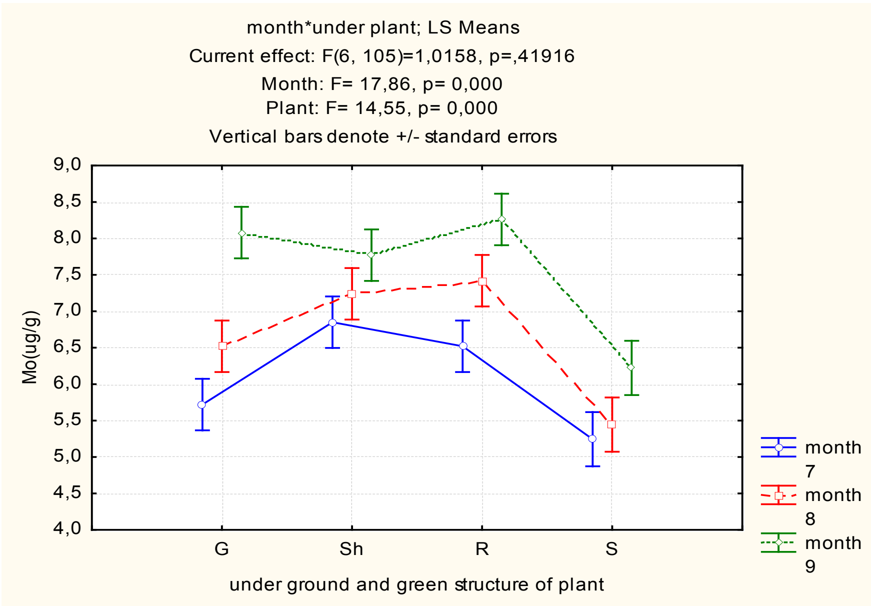

Fig. 12. Accumulation of molybdenum in the soils (S), roots (R), sheaths (Sh) and green (G) above gound parts of *Juncus trifidus* (for detailed explanation see text).

fires, volcanic activity). Yearly, 50 tons of antimony is released into the air from natural processes. In the past 150 tons per year of antimony was released into the air from anthropogenic processes (2005). Nowadays, that amount has increased, totalling up to 1600 tons (Vojteková *et al.* 2013).

Strontium (Sr), Barium (Ba)

Strontium was found in small concentrations in leaves and roots. Higher amounts were found in soil than in the plant iteslf, meaning that the plant did not actively absorb this element (Fig. 16).

Strontium is a relatively common element in the Earth's crust and its prevalence ranges between 260 and 730 mg kg^{-1}. It is likely to concentrate in mafic igneous rocks and in carbonate sediments. Both geochemical and biochemical characteristics of Sr are similar to those of Ca. Strontium possesses lithophilic affinity and is associated with Ca, and to a lesser extent with Mg (Kabata-Pendias and Mukherjee 2007).

Barium (Fig. 17) is a common and ubiquitous element. Its mean content in the Earth's crust amounts to 425 mg kg^{-1}, and ranges from 550 to 668 mg kg^{-1} in the upper continental crust. Barium has a lithophilic affinity and is likely to concentrate in acid igneous rocks and argillaceous sediments, ranging widely in various rocks from 250 to 1200 mg kg^{-1} (Kabata-Pendias and Mukherjee 2007).

Barium occurs naturally in minerals such as witherite, celestine, barite, etc. It is easily absorbed into the environment through mineral digestion and

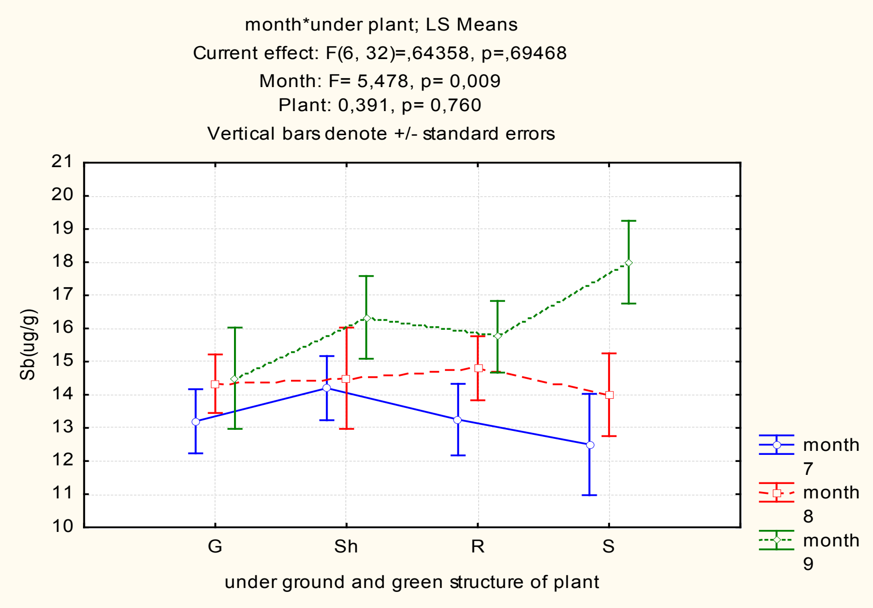

Fig. 13. Amount of antimony was the same in soil and green part of plant. Differences in amount of antimony could be seen during the months, whereas the highest antimony concentrations were found in autumn. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

thus occurs in consentrations of up to several tens of micrograms per litre. This element is also absorbed into the environment as a result of anthropogenic factors, (e.g. waste waters of industrial production of paper, glass, dyes, ceramics etc.) (Orolínová 2009).

Arsenic (As), Titanium (Ti)

In some samples, the concentration of arsenic in aboveground parts of the plant was below the detaction limit of the testing device. Consistently high amounts of arsenic were found in the soil over all months. The plant did not pull out arsenic from the soil into its thallus (Fig. 18).

Arsenic is widely distributed in the environment. It occurs in the Earth's crust at levels between 0.5 and 2.5 mg kg^{-1} and is likely to be concentrated, up to 13 mg kg-1, in argillaceous sediments (Kabata-Pendias and Mukherjee 2007).

Titanium concentration in the soil was high over all months, but roots and green parts of the plant do not absorb it. Thus, there is a wide data range on titanium concentration in green parts of plants, as it was under the detection limit in some samples. Titanium is not easily absorbed by the plant, although it can be found in high amounts in soil (Fig. 19).

Titanium shows strong lithophilic characteristics and is a common element in rock, in the range

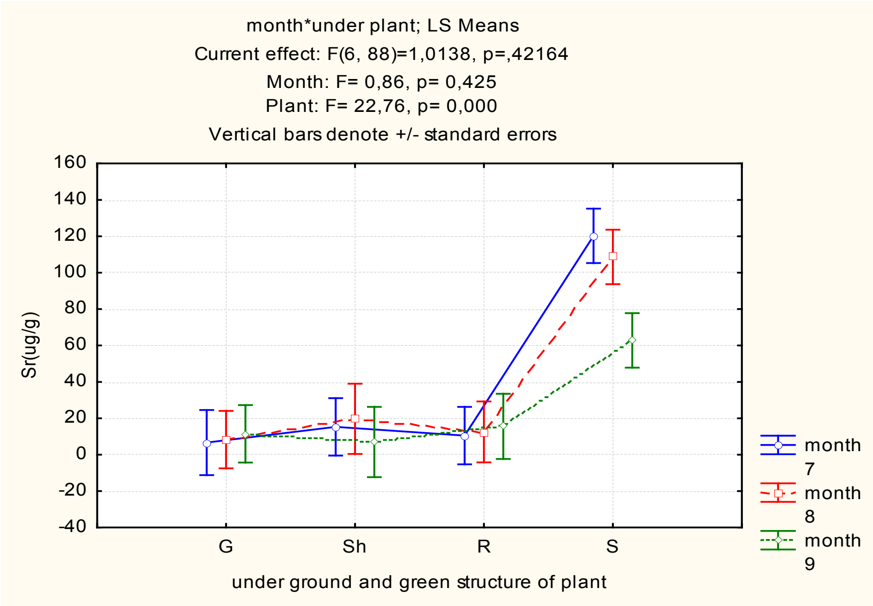

Fig. 14. Accumulation of strontium in the soils (S), roots (R), sheaths (Sh) and green (G) above gound parts of *Juncus trifidus* (for detailed explanation see text).

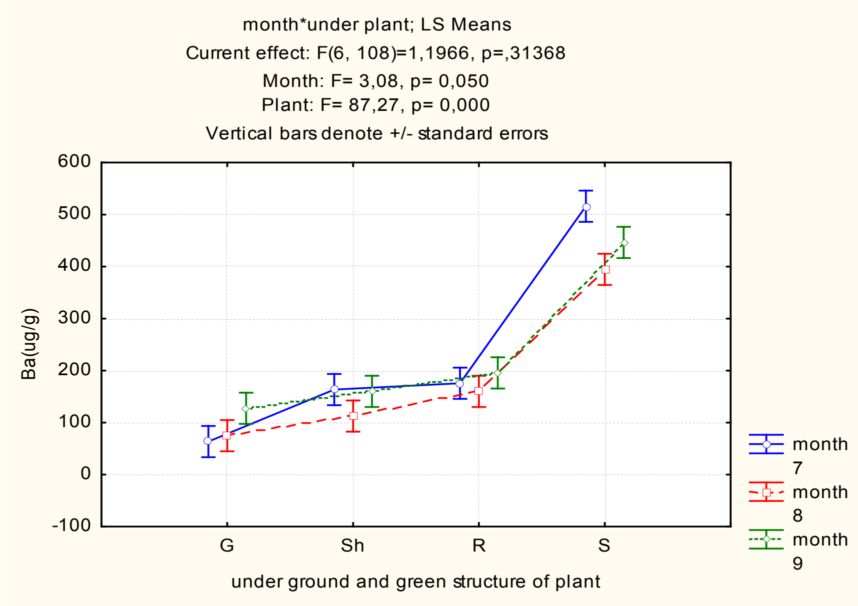

Fig. 15. Barium was found mainly in soil, whereas the plant did not absorb it much into the roots. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

of 0.03-1.4%. Its average abundance in the Earth's crust is 0.4-0.6%. Titanium exhibits variable valences, but in minerals occurs mainly in the tetravalent oxidation state as a major component of oxides, titanates, and silicates (Kabata-Pendias and Mukherjee 2007).

Conclusion

Our research started with sampling in July 2016 and concluded in September 2016, as the sample areas became covered by snowfall. Over those three months we collected 30 plants of *Juncus trifidus* from 10 collection sites. In all, there were 120 samples to analyse, as each plant was divided into into 4 parts (aboveground green part, green part, roots and soil). It was necessary to divide the plants into 4 parts to determine in which part there was the greatest concentration of toxic substance. This made it easier for us to determine how these substances may have gotten into the body of the plant.

Our aim was to prove the existence of specific toxic elements that possibly occur in plants of *Juncus trifidus* and to try to determine their mechanism of occurence. We proved the presence of nineteen elements altogether. Discovery of these elements in the ecosystem helped us to understad how vulnerable this ecosystem is, even when it appears untouched by humans at first glance.

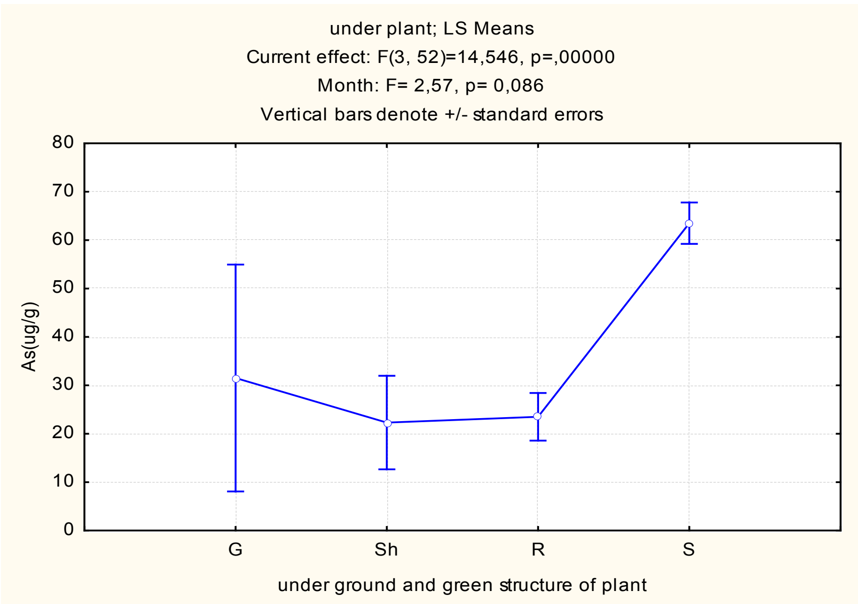

Fig. 16. Accumulation of arsenic in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

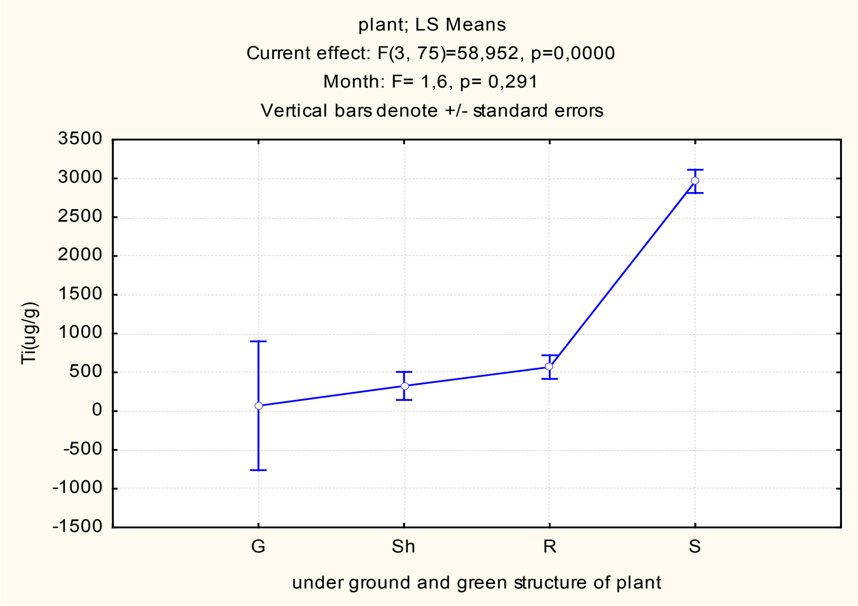

Fig. 17. Accumulation of titanium in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

As previously mentioned, our research coincided with research on toxic substances and the snow vole (*Chionomys nivalis*). As *Juncus trifidus* forms part of its diet, it is reasonable to infer that toxic substances may enter its body through this plant. the Presence of specific elements helped us to better imagine the way these move in an ecosystem, which part of the plant absorbs the highest quantity of which elements, and which elements are important for the plant.

It would be valuable to continue this research and resume collection early in the spring (2017) in order to establish comparative analysis, which could lend weight to our theories regarding mechanism of occurence of these toxic elements and possibly lead to steps toward prevention of the accumulation of these toxic elements in Dolina Bielej vody Valley.

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REPORTS

New records of ectoparasites from passerine birds in the High Tatras of Slovakia

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Abstract. During the summer of 2015, 52 passerine birds, representing 16 genera and 19 species were captured at a field site in the High Tatra Mountains, Tatranská Javorina, Slovakia. These birds were examined for ectoparasites, including chewing lice (Phthiraptera: Ischnocera), fleas (Siphonaptera), flies (Diptera: Hippoboscidae), and feather mites (Acari). A list of host-parasite associations is provided, along with data on parasite prevalence and intensity. Many of the records are known host associations, but two species of lice (one named, one unnamed) represent new host records.

Key words: Passeriformes, Phthiraptera, Ischnocera, Philopteridae, Ricinidae, Siphonaptera, Acari

Introduction

The High Tatras are a mountain range along the Slovakia-Poland border. We surveyed the ectoparasites of birds mist-netted in the High Tatras June-July 2015 at the Institute of High Mountain Biology (University of Žilina), which is situated in the small village of Tatranská Javorina, Slovakia. The site contains mature mixed deciduous and coniferous trees and small open fields with wildflowers, adjacent to a mountain stream. We concentrated primarily on chewing lice, which are permanent parasites that pass all stages of their life cycle on the body of the host (Clayton *et al.* 2015). The collecting method we used is particularly effective for quantifying populations of lice, as we describe below.

Material and Methods

Birds were captured with mist-nets placed on the grounds of the Institute of High Mountain Biology, Tatranská Javorina, Slovakia (49.266° N 20.143° E elevation 1000 m), during June and July of 2015. Each bird was processed on location. Ectoparasites were removed by placing each bird in a "fumigation chamber" or "anethesia jar" for at least 15 min., which is a standard method for removing ectoparasites from live birds (Clayton and Drown 2001). Briefly, a cloth collar was fitted around the neck of each bird and the body of the bird lowered into a wide-mouthed glass jar containing a cotton-ball soaked with chloroform. The chloroform vapors penetrated the bird's plumage and killed ectoparasitic arthropods, which were then collected by removing the bird from the fumigation chamber and ruffling its feathers over a clean white sheet of paper. This method is described in detail by Clayton and Drown (2001). Since the bird's head remained out of the jar during this process, each bird's head was visually examined and any parasites on the head were removed with forceps. This sampling method recovers most lice, as well as other ectoparasites like fleas and flies (Clayton and Walther 1997; Clayton and Drown 2001). However, it is less reliable for the removal of feather mites. We examined the flight feathers of each bird for mites, and preserved a sample of mites from infested birds.

To avoid cross-contamination, birds were held in clean paper-bags prior to fumigation, and the chambers and all working surfaces were carefully cleaned and inspected between birds. All recovered parasites were preserved in 95% ethanol, and are deposited in the Price Institute of Parasite Research (PIPeR) at the University of Utah, Salt Lake City, Utah, USA.

Results

A total of 52 passerine birds representing 16 genera and 19 species were examined (Table 1). Overall, 44.2% (23/52) birds were infested with at least one type of ectoparasite.

Lice

Lice were the most common ectoparasite. In all, 26.9% (14/52) of birds were infested with lice. Ten species of feather lice were recovered (Table 2): four species in the *Brueelia*-complex (*Brueelia* spp., and *Guimaraesiella* spp.) (Gustafsson and Bush 2017), five species of *Philopterus*, and one species of *Ricinus*. All of these genera are already known to be associated with passerines; however, two of the species collected represent new host records: *Philopterus fringillae* ex. *Pyrrhula pyrrhula* (Eurasian bullfinch), and *Philopterus* sp. ex. *Carduelis spinus* (Eurasian siskin).

Bird species	# Birds examined	Lice	Fleas	Flies	Mites •
		# Birds infested (Louse intensity*)	# Birds infested (Flea intensity*)	# Birds infested (Fly intensity*)	# Birds infested
Carduelis carduelis	3	0	0	0	-
Carduelis spinus	4	2 (8-11)	0	0	-
Carpodacus erythrinus	1	0	0	0	-
Erithacus rubecula	2	0	1 (1)	0	1
Fringilla coelebs	4	3 (19-92)	0	0	-
Motacilla cinerea	1	0	1 (3)	0	1
Muscicapa striata	2	0	0	0	1
Parus major	7	0	0	1 (1)	-
Periparus ater	2	0	0	0	-
Phoenicurus ochruros	4	0	1 (1)	0	2
Phylloscopus collybita	2	0	0	0	-
Poecile montanus	4	0	0	0	-
Prunella modularis	4	3 (1-15)	1 (1)	0	-
Pyrrhula pyrrhula	1	1	1 (6)	0	-
Serinus serinus	1	0	0	0	1
Sylvia atricapilla	1	0	0	0	2
Turdus merula	2	1 (160)	1 (6)	0	-
Turdus philomelos	1	0	0	0	-
Turdus pilaris	4	4 (3-89)	0	0	-
Total	**52**	**14**	**6**	**1**	**8**

* Intensity reported as the range in number of parasites infesting individual birds.
• Intensity not reported for mites because many mites remained on the flight feathers after fumigation. The number of birds infested with mites is based on visual examination of the flight feathers of each bird.

Table 1. List of ectoparasites recovered from birds in the study.

Host	Lice *	# Birds infested	Mean intensity (range)
Carduelis spinus	*Brueelia chrysomystris*	1	10.0
	Philopterus sp. *	2	4.5 (1-8)
Fringilla coelebs	*Brueelia kluzi*	1	87.0
	Philopterus fortunatus	3	18.7 (5-32)
Prunella modularis	*Philopterus modularis*	3	6.0 (1-15)
Pyrrhula pyrrhula	*Philopterus fringillae* *	1	7.0
Turdus merula	*Guimaraesiella amsel*	1	160.0
Turdus pilaris	*Guimaraesiella marginata*	4	25.8 (3-87)
	Philopterus bischoffi	1	2.0
	Ricinus elongatus	1	1.0

* New host record

Table 2. Summary of host-louse associations.

The unnamed *Philopterus* was a unique morphospecies found on two different host individuals. No lice in the genus *Philopterus* are known from this host (Price *et al.* 2003), so this may be a new species.

Other ectoparasites

Fleas (Siponaptera) were found on 11.5% (6/52) of the birds, and a single hippoboscid fly (Diptera) was found on one of the 52 birds. Feather mites (Acari) were found on 15.4% (8/52) of the birds.

Discussion

A study of avian ectoparasites by Sychra *et al.* (2011) examined the ectoparasites of passerine birds captured near the Sub-Beskidian Hills of the Czech Republic (49° 34′ N, 17° 59E, elev. 400m). Sychra *et al.* (2011) examined 16 avian species, nine of which were species we also examined in this study: *Fringilla coelebs*, *Parus majer*, *Periparus ater*, *Phylloscopus collybitta*, *Prunella modularis*, *Pyrrhula pyrrhula*, *Sylvia atricapilla*, *Turdus merula*, and *Turdus philomelos*. Sychra *et al.* (2011) observed the following prevalence of parasites: lice = 15.3% (autumn, 2005) and 13.5% (spring, 2007); fleas = 0.8% (autumn, 2005) and 2.9% (spring, 2007); hippoboscid flies = 0% (spring, 2007). In comparison, we observed a higher prevalence of all three of these ectoparasitic insects in our study: lice = 26.9%, fleas = 11.5%, and flies = 1.9%. Many factors could be responsible for these differences, such as differences in locality, season, host species composition, etc. In both studies, however, lice were the most commonly observed ectoparasitic insects.

We found species of lice that are new host records (Table 1), and one may be a new species (*Philopterus* sp. ex. *Carduelis spinus*). Additional taxonomic work is required to make that determination.

In addition to ectoparasitic insects, we found feather mites on 15.4% of the birds in our study, which is probably an under estimate of true mite prevalence. Our estimate is based on examination of feather mites on the flight feathers of the wings, yet mites are known to inhabit other microhabitats on the body of the bird. For example, a single parrot species (*Aratinga holochlora*) was infested with at least 25 species of feather mites, and probably hosted several species of skin mites, nest mites, quill mites, and nasal mites (Perez 1995; 1997). Future surveys that screen more carefully for mites will likely reveal a higher prevalence of mites than we documented.

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