Oecologia
Montana 2016,
25, 39-48

Dust nuisance and its potential influence on *Pieris rapae* and *Maniola jurtina*

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Abstract. In this paper we focused on the usage of *Lepidoptera* species, namely *Pieris rapae* and *Maniola jurtina* as bioindicators of pollution in the town of Ružomberok. We chose three sample plots in the Lisková village, near the pulp factory, and four plots in the control localities Hrboltová and Vlkolínec. The samples of butterfly imagos were collected in June, July and August 2015. The statistical analyses proved there is some pollution of Rb and Mo in the Lisková village caused indirectly by the pulp factory.

Key words: bioindication, *Lepidoptera*, *Maniola jurtina*, *Pieris rapae*, air pollutants, pulp industry, XRF spectrometry

Introduction

The basic idea of bioindication is the fact that some organisms are dependent on certain environmental factors (Carl *et al.* 2004). Bioindication indicates both negative and positive changes of habitat quality. It is not a ground - breaking method of the assessment of environmental changes; in the past, different species of indicator plants have been used in agriculture to show the state of soil or vegetation (Kontrišová 2006).

One of the methods of bioindication is toxicity testing, eventually testing of the accumulation of elements in living organisms. Organisms used by this method (i.e. cumulation organisms) react to changes in the manifestation of an element in the environment at most by morphological or anatomical changes, most often by the reduced growth rate (Kontrišová 2006).

We may assume that insects, especially order *Lepidoptera*, represent a group of fauna suitable for this type of bioindication (Hluchý *et al.* 2007), since they meet some key preconditions: 1) easy identification and surveillance and 2) wide area of distribution (Syaripuddin *et al.* 2015; Hammond 1995). Insects of the *Lepidoptera* order are often used to indicate the state of the environment because of their sensitivity to habitat change, especially to pollution by anthropogenic activity (Hammond 1995).

Individuals of the genus *Pieris* spp., specifically *P. napi*, *P. rapae* and *P. brassicae* have a relatively wide area of distribution (Emmel 1973). For comparison, *Maniola jurtina* individuals are more sedentary, i.e. with a smaller area of distribution, populating one habitat (Brakefield 1982). We may therefore assume that imagos of *Pieris spp.* from different locations not too far from each other will contain approximately the same proportions of accumulated elements, while the values for *M. jurtina* will differ depending on the location of sampling.

For our study we have chosen the surroundings of the town of Ružomberok as a model locality, which is characterized by long term industrial pollution from the pulp factory Mondi SCP. Pollutant emissions are emitted to the atmosphere from the factories production and subsequently enter all parts of the environment, accumulate in plants and animals and finally by entering the food chain negatively affect human health.

One of such elements is for example sulphur dioxide SO_2, which enters the atmosphere by burning substances containing sulphur. In pulp production such substances are most often coal and oil. Besides the negative impact of this compound on the environment (glasshouse effect, acid rains), there is a considerable influence on human health by sedimentations in breathing tubes. Another element of this kind is chlorine. This study is focused on the two elements and their toxic compounds used in pulp production in whitening.

The main aim of this study is to determine the impact and accumulation of pollutants (their identification and concentrations) from the pulp production factory Mondi SCP in the imagos of the chosen bioindicator insect group of diurnal butterflies as in the feeding plants of their larva - caterpillars in relation to measured pollutants and meteorological data from the area.

To meet the main aim, we have chosen these sub-aims:

- To choose and characterize the potentially most polluted locations in different distances from the source of pollution.
- To gather a sufficient number of samples of the chosen groups of insects and their feeding plants from the chosen locations. To find out and compare the abundancy of butterfly species for the purpose of faunistics.
- To identify and determine the concentrations of pollutants in imagos of the chosen butterfly species as well as in the feeding plants of their larva.
- To add measured meteorological data to our data and assess their impact on the pollution in the region.

Material and Methods

Sampling

We chose three villages differently situated to the source of pollution to assess its impact - Lisková (closest to the assumed source of pollution), Hrboltová (west of the assumed source of pollution, i.e. against the prevailing winds) and Vlkolínec (south of the assumed source of pollution) (Fig. 1).

Three meadows were chosen in Lisková (marked L1, L2 and L3), two in Hrboltová (marked H1 and H2) and two in Vlkolínec (marked V1 and V2) (Table 1). Meadows on opposite sides of the villages in relation to Mondi SCP a.s. were chosen in Vlkolínec and Hrboltová. Meadows were selected in a transect going from south to north through Mondi SCP a.s. in Lisková, while the sampling plot L3 is located in a slightly higher altitude than L1 and L2.

Imago sampling

The species *P. rapae* and *M. jurtina* (Fig. 2) were chosen as model organisms for this study. These species were chosen for their different environmental demands. While *Pieris spp.* are ubiquist, mobile species with an extensive area of the individual; *M. jurtina* is a sedentary species, whose individuals tend to stay on a relatively small area for their whole lifespan.

Capturing of imagos with a butterfly net took place in the months of June, July and August 2015. Sampling was most intensive in July and August when it was accomplished approximately every week. Sampling from all sampling plots took around one and a half day, approximately one and a half hour each plot. The days on which the samples were collected: 26.6., 17.7., 22.7., 23.7., 31.7., 1.8., 5.8., 6.8., 12.8., 13.8., 20.8., 21.8., 30.8., 31.8.

The sampling took place between dusk and dawn, under the conditions of warm weather (appr. 17 - 30˚ C), relatively clear sky and preferably no wind. We used a modified transect method on individual plots, until a butterfly was spotted and captured. Afterwards we continued the transect on the place where the butterfly was spotted. We repeated the transect till there was no individual spotted along it.

Individual imagos were inserted in paper envelopes marked with the date and place of capturing. Species were determined later and imagos were grouped by species, date and sampling plot.

Sampling of feeding plants

Sampling of feeding plants took place in September 2015 in Lisková and Hrboltová. Feeding plants of *M. jurtina* were sampled, namely *Bromus erectus* and *Poa pratensis*.

Sample preparations

The feeding plants and imagos were dried in Memmert IF160plus at temperature of 70˚ C for seven hours. Dried samples were hand grinded to a homogenised powder. This powder was submitted to spectral analysis.

Since the abundance of individuals on certain plots was low, it was not possible to analyse certain samples individually. After the individual analysis we mixed the samples according to a location to assess not only the effects of climate on concentrations of pollutants in samples, but also the effect of the location.

We used XRF spectrometer DELTA CLASSIC (US) able to detect and determine the following elements: S, Pb, Fe, Mn, Cu, K, Ca, zn, Mo, Cr, Ba, Rb, Sr, Ti, Zr, Cl, As, Co, Cd, Zn, I.

The analysis resulted in a Microsoft Excel table. Each sample was described by its species, date of sampling and location. We reclassified and modified this table to simplify it for further statistical analysis. Only average values for each sample were included. Elements that were not present in the sample in an amount enabling detection were excluded.

The following meteorological data were also added to the table: the concentration of CO_2, O_3, dust (expressed by PM_{10} values) and TRS (total reduced sulphur). We only included the average rate for the month, in which the larva developed.

Statistical methods

A statistical software (STATISTICA 10.1) and Microsoft Excel were used for statistical analyses. In this software we made a correlation matrix and determined the eigenvalues for several factors influencing variance in our data. We determined factor scores

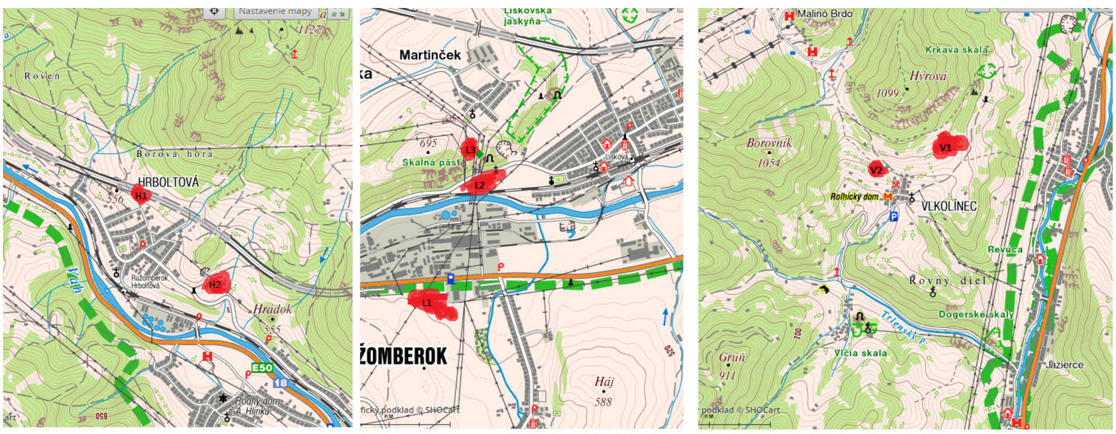

Fig. 1. Map of the sample plots. From left: Hrboltová, Lisková and Vlkolínec. Source: www.mapy.hiking.sk (2016).

	Sample plots				
	Distance from Mondi SCP (km)	Altitude (m a.s.l.)	Orientation (degrees)	Longitude	Latitude
L1	0.22	490	180	19.330495	49.077903
L2	0.67	482	0	19.333092	49.084979
L3	1.03	572	0	19.335152	49.088239
H1	7.08	543	290	19.246489	49.108408
H2	6.20	533	290	19.254278	49.100611
V1	5.36	803	200	19.284490	49.042597
V2	5.88	739	200	19.276776	49.041190

Table. 1. Characteristics of chosen sample plots.

and eigenvectors (Table 4) to show how the factors influenced each sample and each measured element.

The eigenvectors and environmental data (meteorological data, data describing the sample plots) then underwent ANOVA (analysis of variance) to determine whether each factor had some significant impact on the data.

Results

A total of 482 imago samples was collected during the study. The species of *P. napi* and *P. brassicae* were excluded from further parts of the study because of their low abundance. The species *P. rapae* were chosen as the representative mobile species.

We can see that the abundance of *M. jurtina* individuals was significantly lower at the sites in Lisková, compared to Vlkolínec and Hrboltová. The opposite is true for the individuals of *P. rapae*, which were more abundant in Lisková than in other sites (Fig. 3).

The matrix below (Table 2) was made by modifying the Microsoft Excel table, which the XRF Spectrometry device had created. A similar matrix presented in Table 3 was created for the feeding plants collected in Lisková and Hrboltová.

Values of carbon dioxide, ozone and PM_{10} (indicating dustiness) were included only in the samples collected in Lisková, were the values were known. Total reduced sulphur was included only in Lisková and Hrboltová.

The graphs in Fig. 4 and Fig. 5 show the amounts of elements for each sample plot and both species. We may see that there is a significant increase in all elements in the samples of *M. jurtina* from the sample plot Hrboltová 2. We could assume that the reason for this is the high abundance of butterflies at this sample plot; however, Fig. 3 shows that the abundance of *M. jurtina* butterflies at Hrboltová 1 and Hrboltová 2 are the same and the elements in Hrboltová 1 do not accumulate in such high amounts as in Hrboltová 2.

We can also see that butterflies of *P. rapae* species accumulated more elements in the sample plots other than Lisková, despite the fact that their abundance was higher in Lisková than in other sample plots.

The next step was the statistical analysis itself. We produced a correlation matrix and eigenvalues for each factor, as well as eigenvectors and factor scores (Table 3).

The dataset underwent a series of One-way ANOVA tests to see which variables correlate with the factors. In the following section we present only graphs to those ANOVA tests, which were significant (i.e. p was less than 0.05). Abbreviations for localities in Fig. 6 - 12: L - Lisková, H - Hrboltová, V - Vlkolínec.

Factor 1

This factor is responsible for 49% of the variance in our data. It is connected with the increased accumulation of biogenic elements, such as P, K, Ca. ANOVA does not prove any correlation with local-

Fig. 2. *P. rapae* (left) and *M. jurtina* (right). Photo by M. Vojtíček. Source: www.lepidoptera.cz (2016).

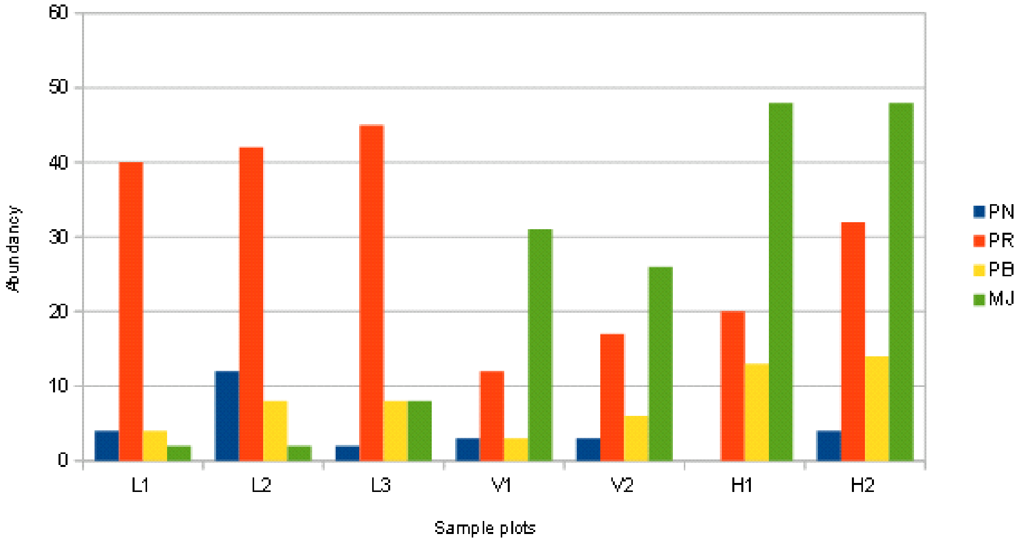

Fig. 3. A graph of the abundance of collected imagos on sample plots.

ity (p = 0.65914). Instead, it seems it is a result of each species different ability to accumulate elements in general (p = 0.14365).

Factor 2

This factor is responsible for 12.5% of the total variance in our data. Mo, Rb and Pb increase along this factor. There is no correlation between Factor 2 and locality, or any other of the chosen variables. This means that along this factor the species of butterflies accumulate Mo, Rb and Pb in the same manner in all of our sample plots.

Factor 3

This factor is responsible for 10% of the total variance in our data. It results in an increase of Ba and Cu on one side, and Rb on the other. As we can see in Fig. 6, the effect of this factor

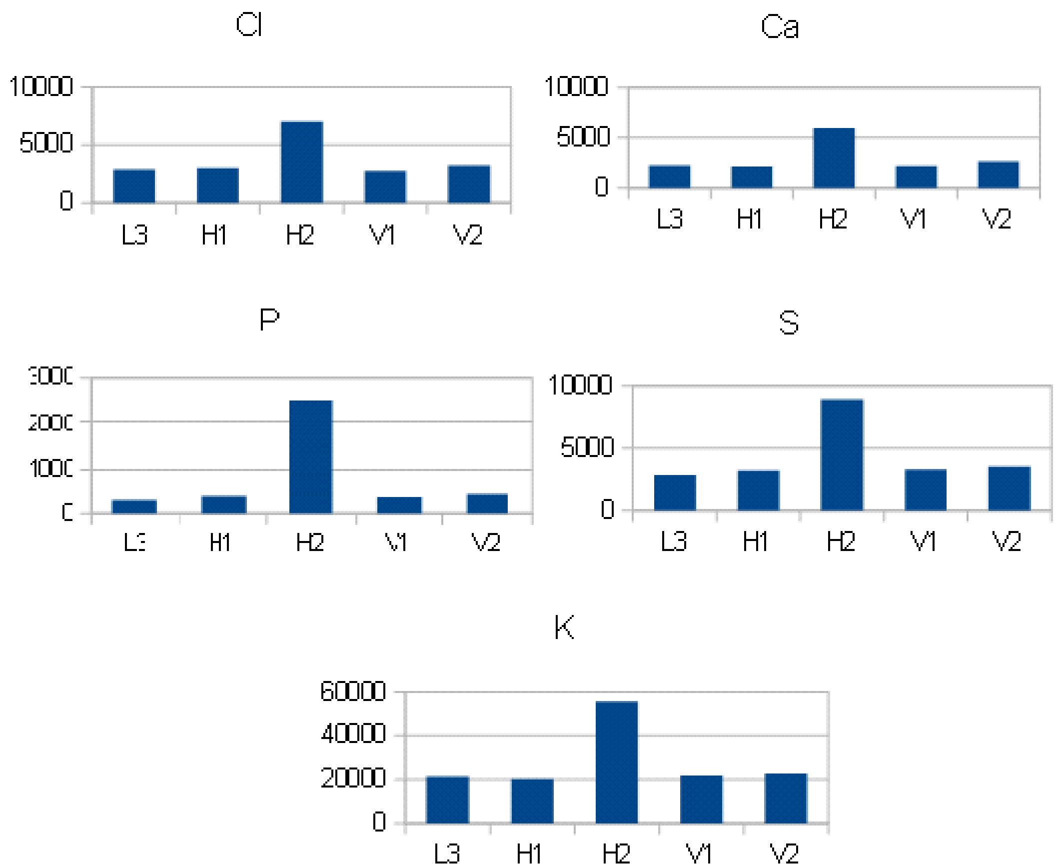

Fig. 4. Graphs for species *M. jurtina*. The total amounts of accumulated elements for each sample plot.

Season	Species	Distance	Orientation	Altitude	PM_{10}	TRS	O_3	CO_2	Locality	P	S	Cl	K	Ca	Cr	Mn	Fe	Cu	Zn	Rb	Mo	Ba	Pb
June	PR	0.5	0	490	21.3584	0.7934	25.714	399	L	1452	5543	4343	41191	4337	53	156	640	56	539	21	7	92	20
July	PR	0.22	180	490	21.3584	0.992	29.4851	404	L	1178	4696	3871	38841	3173	39	127	431	54	492	18	7	54	13
July	PR	1.03	0	572	21.3584	0.992	29.4851	404	L	2078	6592	6741	45594	4945	60	190	1720	47	416	19	5	112	14
July	PR	0.67	0	482	21.3584	0.992	29.4851	404	L	3326	10197	6981	71863	7757	91	222	823	65	431	16	7	151	15
July	MJ	1.03	0	572	21.3584	0.992	29.4851	404	L	1387	6140	4393	43367	4263	59	166	389	58	648	14	7	162	14
Late July	MJ	7.08	290	543	-	90.8095	-	-	H	600.3	4328.7	3020	32047	3429	70	114.3	333	58	655.7	17	7	115	15
Late July	MJ	5.36	200	803	-	90.8095	-	-	V	1381	7985	5075	47859	5030	71	188	683	76	672	18	6	144	14
Late July	MJ	5.88	200	739	-	90.8095	-	-	V	1501	7327	5405	50753	9090	70	177	800	80	563	16	8	116	19
Late July	PR	1.03	0	572	23.9681	0.992	29.4067	416	L	2397	7761	5116	53282	4471	69	171	537	49	539	19	7	90	14
August	PR	0.22	180	490	23.9681	1.0452	29.4067	416	L	1216	5103	3861	38846	3445	44	138	489	66	480	15	6	68	18
August	MJ	7.08	290	543	-	2.9911	-	-	H	811	4935	4120	34933	3329	51	139	327	58	541	16	8	113	20
August	MJ	6.2	290	533	-	2.9911	-	-	H	549	4674	3327	31713	2970	41	131	255	57	616	15	5	118	15
August	MJ	5.36	200	803	-	-	-	-	V	618	4289	2965	30052	2858	35	128	246	51	615	14	5	93	11
August	MJ	5.88	200	739	-	-	-	-	V	887	4569	3897	35069	3436	45	136	288	63	614	15	7	105	15
August	PR	6.2	290	533	-	2.9911	-	-	H	1540	6073	4586	39131	3929	58	145	372	47	481	17	7	78	15
August	MJ	7.08	290	543	-	2.9911	-	-	H	371	3515	3611	26805	2365	27	100	257	51	474	15	5	86	15
August	MJ	6.2	290	533	-	2.9911	-	-	H	925	4644	4211	32014	3344	45	143	304	41	389	15	6	119	13
August	MJ	5.36	200	803	-	-	-	-	V	618	4289	2965	30052	2858	35	128	246	51	615	14	5	93	11
August	MJ	5.88	200	739	-	-	-	-	V	822	4189	3918	35203	8653	45	148	702	65	522	16	6	104	17
August	PR	0.22	180	490	29.5478	1.0452	29.4067	416	L	1955	6961	4653	48423	4980	62	172	615	61	446	18	6	108	18
August	PR	0.67	0	482	29.5478	1.0452	29.4067	416	L	2321	7418	5091	50939	4217	79	170	877	64	472	20	9	88	14
August	PR	5.36	200	803	-	-	-	-	V	3255	10423	6797	63125	5321	74	190	434	52	351	11	5	129	14
August	PR	5.88	200	739	-	-	-	-	V	2069	6582	4548	45859	7030	62	209	1278	50	379	17	8	124	11
August	MJ	7.08	290	543	-	2.9911	-	-	H	3087	11140	9349	67395	7714	145	277	1132	54	501	17	4	179	0.1
August	MJ	6.2	290	533	-	2.9911	-	-	H	1431	5834	5903	37984	4764	73	195	504	52	447	15	8	145	17
August	PR	1.03	0	572	29.5478	1.0452	29.4067	416	L	2588	9400	6717	57810	5477	74	188	515	35	358	15	8	126	18
Late August	PR	0.67	0	482	29.5478	2.9911	29.4067	416	L	2505	8329	5831	51053	7459	64	170	512	41	411	16	5	115	14
Late August	MJ	7.08	290	543	-	2.9911	-	-	H	1866	8027	7923	43607	5425	78	224	526	89	338	15	8	160	16
Late August	MJ	6.2	290	533	-	2.9911	-	-	H	950.7	5528	6043	32889	4019	56	162.3	441.6	63	360	14	6	122	13
Late August	MJ	7.08	290	543	-	2.9911	-	-	H	2285	8092	6490	53542	4646	84	196	577	57	491	17	9	116	10

Table 2. The matrix for butterflies used for statistical analyses (amount of elements measured in ppm).

Sample	S	Cl	K	Ca	Cr	Mn	Fe	Zn	Rb	Sr	Mo	Ba	Pb
Plants L2	408	2110	9289	8602	17	81	548	30	11	17	5.6	41	11
Plants L3	523	2435	7998	8408	28	89	1012	36	12.5	12	5.8	50	13
Plants H1	381	432	1047	2823	33	78	840	37	11.6	2.9	6.7	44	12
Plants H2	475	451	2158	2630	30	119	668	34	12.7	2.9	6.6	37	15

Table. 3. The matrix for feeding plants of butterflies (amount of elements measured in ppm).

on different species in this study is relevant (p = 0.00002). The accumulation of relatively more Rb than Ba and Cu is apparent for the species of *P. rapae*. For comparison, the samples of *M. jurtina* contained Ba and Cu instead of Rb.

The effect of different sample plots is also significant. Fig. 7 shows that imagos of any species sampled at Lisková tend to accumulate more Rb compared to samples from both Hrboltová and Vlkolínec.

Factor 4

This factor is responsible for 9% of the total variance in our data. It mainly affects the accumulation of Zn, Rb and Pb. No significant correlation was found between this factor and the surveyed variables. It seems that neither locality, nor meteorological variables do have effect in this factor.

Factor 5

This factor is responsible for 5.7% of the total variance in our data. Amounts of accumulated Ca, Fe, Mo and Zn increase along this factor. However, no significant correlation was found between this factor and the difference between species, sample plots or climatic variables. The accumulation of Ca, Fe, Mo and Zn along this factor is not influenced by species, location or weather.

Factor 6

This factor is responsible for 4.5% of total variance in our data. Zn, Mo, Pb and biogenic elements, for example K and Ca, increase along this factor. There is a strong correlation between factor 6 and the location of the sample plots (p=0.00298, as seen in Fig. 8). This factor also correlates significantly with the altitude and orientation of the sample plot (Figs.

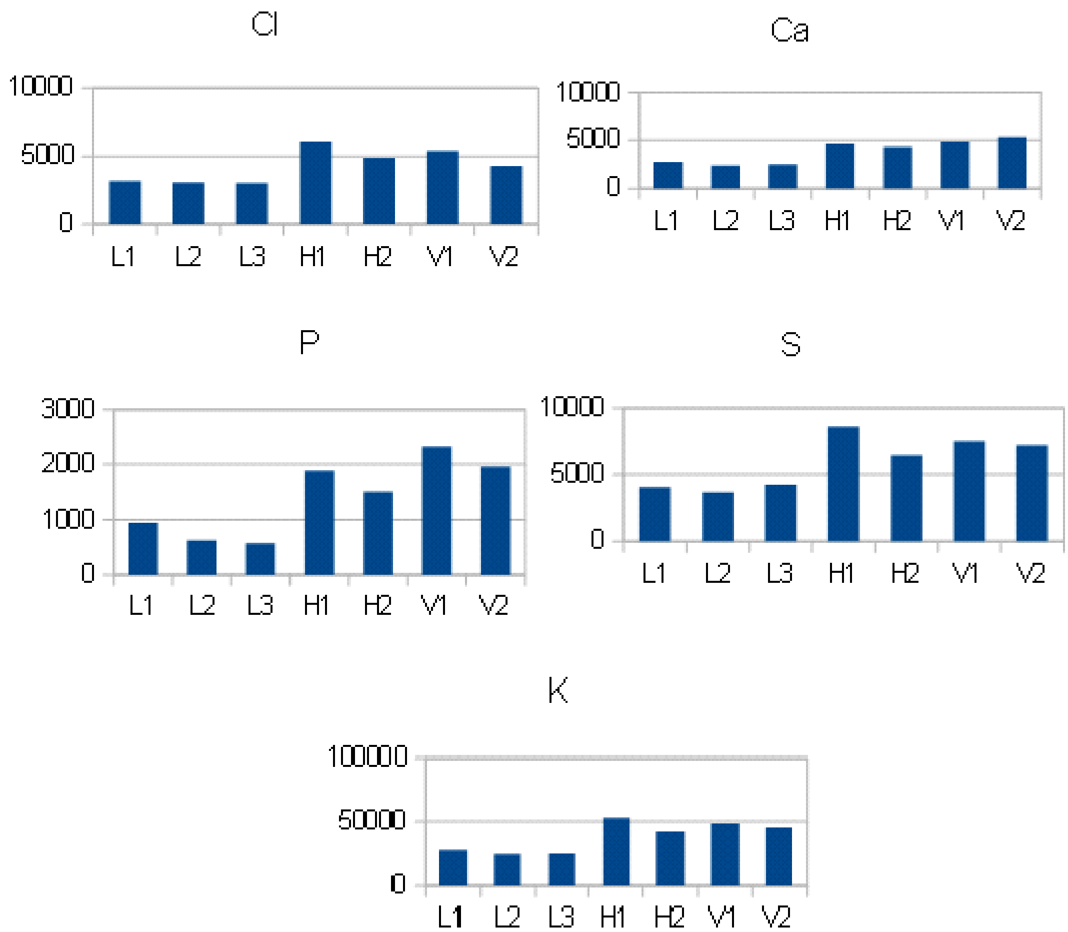

Fig. 5. Graphs for species *M. jurtina*. The total amounts of accumulated elements for each sample plot.

	Eigenvalue	% Total - variance	Cumulative - Eigenvalue	Cumulative - %
1	6.87	49.09	6.87	49.09
2	1.75	12.53	8.62	61.62
3	1.40	10.05	10.03	71.68
4	1.27	9.07	11.30	80.76
5	0.80	5.75	12.11	86.52
6	0.63	4.56	12.75	91.08
7	0.46	3.28	13.21	94.37
8	0.35	2.50	13.56	96.87
9	0.21	1.51	13.77	98.39
10	0.09	0.67	13.86	99.06
11	0.06	0.43	13.92	99.49
12	0.03	0.26	13.96	99.76
13	0.02	0.13	13.98	99.90
14	0.01	0.09	14.00	100.00

Table. 4. Eigenvalues of correlation matrix, and related statistics.

9 and 10). More Mo was accumulated in Hrboltová, compared to Lisková and Vlkolínec.

Factor 7

This factor is responsible for 3.2% of the total variance in our data. Ca, Cu and Ba amounts in imagos increase along this factor. No correlation was found between this factor and the surveyed variables. Therefore, the increase of Ca, Cu and Ba along this factor cannot be explained by different species, locality or weather.

Factor 8

This factor is responsible for 2.5% of the total variance in our data. It affects mainly the accumulation of Ca, Ba and Pb. ANOVA tests showed that there is a slight correlation between this factor and the location of each sample plot (p=0.05669; Fig.11). The butterflies sampled in Vlkolínec increase more Ca and the butterflies in both Lisková and Hrboltová accumulate Ba and Pb instead of Ca.

Factor 9

This factor is responsible for 1.5% of the total variance in our data. The accumulation of Fe, Ca and Rb increase along this factor. A correlation was found between this factor and locality (p=0.04822). Samples from Hrboltová accumulated more Ca and Rb than samples from Lisková and Vlkolínec, which accumulated more Fe instead (Fig. 12).

Discussion

The effect of atmospheric pollution on living organisms has been dealt with in several studies. Lodenius *et al.* (2009) presented results suggesting that ash fertilization has little or no impact on cadmium accumulation in beetles. On the other hand, in west Hungary, Horváth *et al.* (2001) studied bark - dwelling and foliage - dwelling spiders at an area with high imission load and in a control area and their results suggest that pollution has direct and indirect influence on the diversity and composition of the spider populations. Van Ooik *et al.* (2007) discovered that the growth of larvae of *Epirrita autumnata* is reduced in a polluted environment, since the larva has to invest more energy and nutrients into the immune response. The idea of indirect influence of pollution on the populations of living organisms is also supported by the findings of Gallo (1997), who studied the community structure of ca-

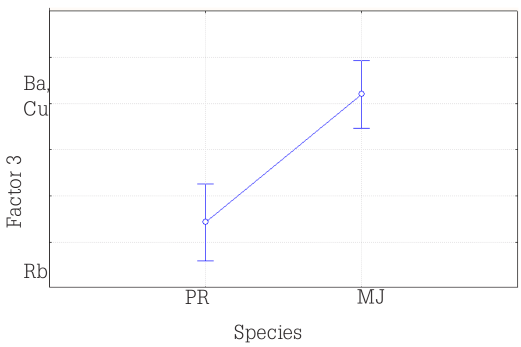

Fig. 6. ANOVA graph of the effect of factor 3 on species of *P. rapae* (PR) and *M. jurtina* (MJ) (F (1, 28) = 26.183, p = 0.00002).

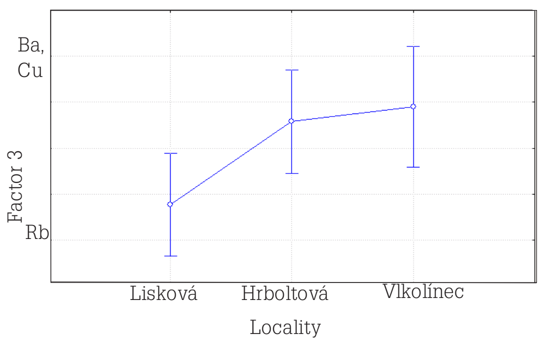

Fig. 7. ANOVA graph of the effect of sample plot location on the accumulation of Rb, Ba and Cu (F (2, 27) = 4.0638, p = 0.029).

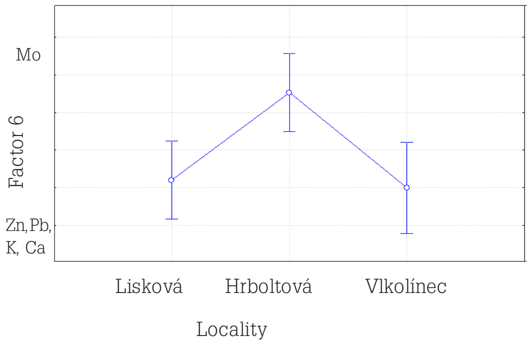

Fig. 8. ANOVA graph showing the correlation between different sample plots and accumulation of Mo, Zn, Pb, K and Ca (F (2, 27)=7.2702, p=0.003).

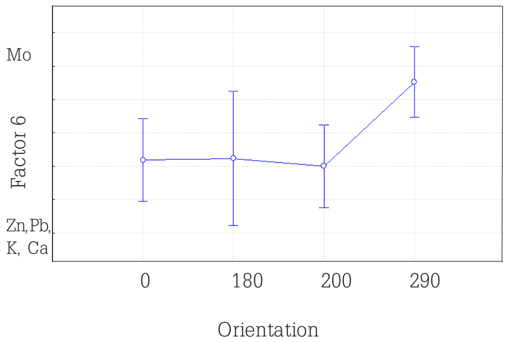

Fig. 9. ANOVA graph showing the correlation between altitude and factor 3 (F (3,26)=4.6681, p=0.01).

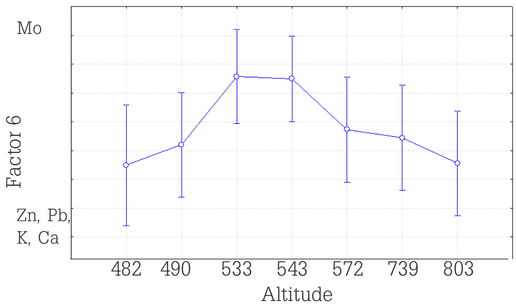

Fig. 10. ANOVA graph showing the correlation between orientation and accumulation of Mo, Zn, Pb, K, Ca. (F (6, 23) = 2.4124, p = 0.05).

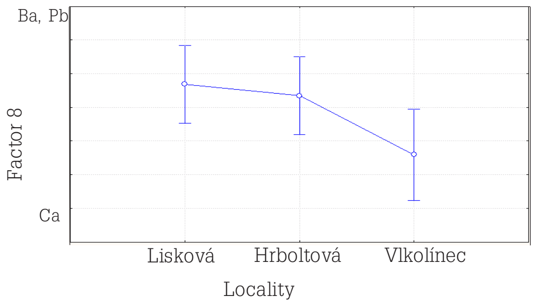

Fig. 11. ANOVA graph showing the correlation between the location of sample plots and accumulation of Ba, Pb and Ca (F (2, 27) = 3.1980, p = 0.056).

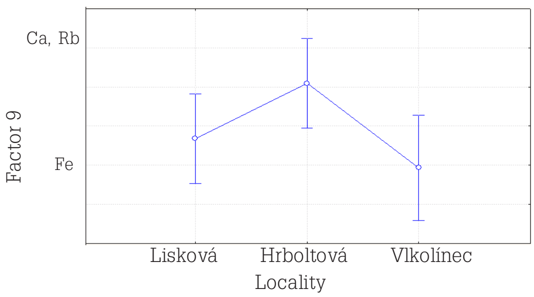

Fig. 12. ANOVA graph showing the correlation between locality and the accumulation of Ca, Fe and Rb (F (2,27) = 3.3994, p = 0.048).

rabid species. He discovered a temporal difference between populations and attributed them to the competitive advantages of an invasive species in a polluted environment. Invertebrate orders have also been used to assess the quantity of elements in the environment (Magalhaes *et al.* 2013). This study serves to further emphasize the role of invertebrates (specifically order *Lepidoptera*) in environmental quality assessment.

Our data show that there are several differences between the sample plots in Lisková, Hrboltová and Vlkolínec concerning the accumulation of elements by living organisms, namely the accumulation of Rb (Fig. 7), Mo (Fig. 8) and Pb (Fig. 11).

The strongest correlation (p = 0.02865) was between Rb and locality. Fig. 7 depicts the fact that our butterfly samples accumulate Rb at the highest rate in Lisková and the lowest one in Vlkolínec. Since Hrboltová is situated near the main road to the town of Ružomberok, but also not so exposed to the impact of the factory, and at the same time the accumulation of Rb, Ba and Cu does not differ in the imagos of butterflies from Lisková and Vlkolínec, we may assume that this phenomenon (the increase of Rb at the expense of Ba and Cu in imagos) is specific for the locality of Lisková; furthermore, the reaction of *P. rapae* is stronger than the reaction of *M. jurtina*.

We see in Fig. 7 that samples in Vlkolínec tended to accumulate less Rb than samples from Hrboltová. However, Hrboltová plots are the farthest from the factory, even farther than Vlkolínec, and since the distance from the factory seems to be the main variable influencing the differences, Hrboltová samples should have accumulated less Rb than those ones in Vlkolínec. The real situation can be explained by the fact that the sample plots in Vlkolínec are separated from the factory by hills and lie perpendicularly to the prevailing winds.

Nevertheless, it is obvious that the accumulation of Rb is typical for the samples from Lisková. Although Rb is present in the Earth crust more often than copper, lead, or zinc, in contrast to them, it does not produce minerals, and is produced only in small amounts. Also, rubidium is quite reactive (Butterman and Reese 2003), therefore it should not stay in the environment for long periods of time. However, rubidium has a ionic radius similar to potassium and is often a substitute of it in cases of low potassium supply, in order to prevent the accumulation of amino acids and amides (Steward 1963). Its presence can also be beneficial, as was proved in the case of rat tissues, where it prevented lesions in kidneys at a potassium - deficient diet (Comittee on Mineral and Toxic Substances in Diets and Water for Animals 2005). Rb often occurs as an impurity of K (Butterman and Reese 2003).

The findings of Nyholm and Tyler (2000) shows that acid rains cause the leaching of K^+, which is subsequently substituted by Rb; i.e. plants and fungi accumulate Rb instead of potassium. Also, Rb is not eliminated throughout the food web. We may assume that the emissions from Mondi SCP a.s. produce slightly acidic rains, which cause the leaching of potassium. K is then substituted by Rb in plant tissues and rubidium enters the body of butterflies through their feeding plants.

When we look at the effect of species on the accumulation of Rb, we see that *P. rapae* species are much more sensitive to the factor influencing the accumulation. This can be explained in several ways. One explanation is simply the different physiology of the two species. Different species accumulate elements and compounds differently and it seems to be a legitimate explanation. There is also the fact that larvae of the two species have different diets. Perhaps the reason behind the correlation between Factor 3 and species is not the different physiology of the butterflies themselves, but of their feeding plants. Finally, we can see in Fig. 3 that the number of *P. rapae* butterflies sampled in Lisková was much higher and *M. jurtina* was fairly rare in Lisková plots; this could also explain higher amounts of Rb in *P. rapae*.

The accumulation of Mo also correlated with locality ($p = 0.00298$), as we can see in Fig. 8. We can also see it correlates with orientation ($p = 0.00971$) and altitude ($p = 0.05898$), which are variables that describe localities more precisely. It shows us that samples in Hrboltová accumulate Mo instead of Zn, Pb, K and Ca.

Molybdenum is an element present in soils as a micronutrient. It is less soluble in acid soils and more soluble in alkaline soil. Its availability to plants depends on the pH of the soil and also on drainage (www.lenntech.com 2016). This could explain the correlation of Mo accumulation to altitude, since localities with lower altitude have faster runoff.

We see a significant correlation between the accumulation of Pb and locality in Factor 8. Since the Vlkolínec sample plot is the one that does not accumulate Pb and it is known that Pb can act as a substitute for Ca in human organisms (Goldstein 1993), we may assume that the sample plots in Hrboltová and Lisková - which are closer to the main roads - are affected by car transportation and the butterflies from these localities substitute Ca with Pb.

It is interesting to see that the population of *M. jurtina* was not very abundant in Lisková; in fact, only a few individuals were captured on the three plots from there. Yet the feeding plants of their larvae, mainly grasses from *Poaceae* order, are present in these plots as well as in Vlkolínec and Hrboltová. There must be another reason for the low numbers of *M. jurtina*, especially when we see in Fig. 3 that the populations in Vlkolínec and Hrboltová were quite abundant. We may assume that the butterfly *M. jurtina* is more sensitive to habitat quality and that it avoids meadows close to Lisková.

Another difference between the sample plots can be seen in Fig. 4 and 5. We could have predicted that the amount of accumulated biogenic elements (Ca, K and P) would be higher for both species at localities other than Lisková and the amount of S and Cl, emitted from the pulp production, would be higher at Lisková; this should be true at least for M. *jurtina*, since it is a sedentary species and spends most of its lifetime on one place, thus it should be able to reflect the different element composition more accurately. As for *P. rapae* butterflies, the amounts should be approximately the same, since it is an ubiquist. However, Fig. 4 and 5 do not show the expected results. We can see that for *M. jurtina*, the accumulation of each element was higher in Hrboltová 2. It is not because of the higher number of samples collected from there, since the number of samples was roughly the same as in Hrboltová 1. It also can not reflect the negative influence of the factory, because in that case, the samples of butterfly imagos from Hrboltová 1 would accumulate elements in a similar manner. There is the fact that, compared to Hrboltová 1, Hrboltová 2 is situated farther from the main road to the town of Ružomberok; however, in this case the accumulation should be similar to the plots in Vlkolínec, where the main road to the town of Banská Bystrica is even farther. The case of *P. rapae* butterflies also cannot be influenced by the factory. The butterfly samples from Lisková accumulated less elements than the samples from Hrboltová and Vlkolínec. If it were because of the impact of the factory, then at least the accumulation of S and Cl should be increased in Lisková.

To summarize our findings, our data have shown that there are differences between the accumulation of certain elements (most notably Rb, Mo and Pb) in imagos of butterfly species *P. rapae* and *M. jurtina*. The accumulation of Rb is different in Lisková and in the other two sample plots, and can be attributed to the influence of K^+ leaching, which can be the result of acid rains. The accumulation of Mo can be attributed to the pH levels of soils and the accumulation of Pb is connected to the traffic. Data on abundancy presented in Fig. 3 show that *M. jurtina* butterflies occur less frequently in Lisková than in Vlkolínec or Hrboltová. These data agree with the fact that *M. jurtina* species is more demanding on the quality of the environment than the butterflies of *P. rapae* species.

We may assume that these findings support the idea of the Mondi SCP a.s. pulp factory influence, since pulp production is known for its high sulphur oxides emissions (RÚVZ 2006). These do not result in the higher accumulation of S and Cl in butterfly imagos, as we originally expected, but rather in an indirect influence: Emissions of S are responsible for the lower pH values of the soils in Lisková.

Conclusions

The sampling for this study lasted from June to August 2016. A total amount of 482 imago samples of species *M. jurtina*, *P. rapae*, *P. napi* and *P. brassicae* has been collected with a butterfly - catchin net using a modified - transect method.

We were able to determine the concentration of elements accumulated in the imagos and statistically process this data.

Our finding prove a mixed effects source of pollution in the region. One of the sources is the main east - west road, hence the intense traffic. Another source of pollution is Mondi SCP a.s., altough our study proved only an indirect impact on the accumulation of certain elements in butterfly imagos. The emission of sulfur compounds results in an acid environment, specifically the soil, from which Rb and Mo enter the bodies of butterflies through the food chain. The accumulated elements last long enough in butterflies to endure from larval stage to imago stage.

Our study provides further evidence for the high suitability of invertebrate orders (and specifically order *Lepidoptera*) for bioindication of pollution and element accumulation.

For the reasons mentioned above we suggest further study of the effect of TRS emissions on the pH level of soils. This way we could exclude other sources of pollution possibly responsible for the increased levels of Rb and Mo in butterfly imago and also confirm the environmental impact of a pulp and paper production factory.

Acknowledgements

This work was partly supported by Structural funds of EU - ITMS - 26210120006 and ITMS - 26210120016.

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