Genotoxic effects of transboundary pollutants in *Pinus mugo* Turra in the high mountain habitats of the Western Carpathians

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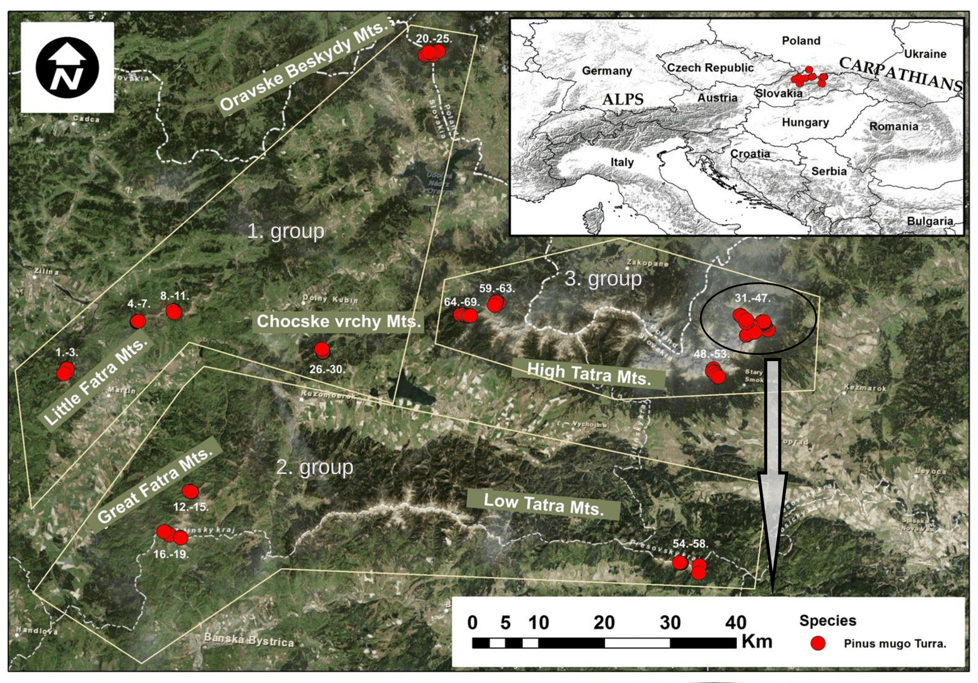
Introduction/aims

The main objective of this study was detect the rate of contamination of mountain and alpine habitats of Slovak mountains. Evaluate the overall genotoxicity of the environment by using pollen analysis (Pollen grain abortion assay) and its relationship to the parallel monitoring of selected heavy metals - Pb, Cd characterizing the prevailing imission load in these ecosystems was part of this goal. Bacause this method has so far been mostly used to assess the environment near industrial sources, factories and roads, so the aim of this study was highlight its applicability for pollutant evaluation in mountain and alpine environment, where only impact of pollutants from long-range transport can be expected. It was crucial to verify that the Pollen grain abortion assay was sensitive enough to indicate contamination from distant sources. Swiss mountain pine (*Pinus mugo* Turra.) was used such as suitable bioindicator. Collection of soil and plant samples (pollen, needles) was done during the 2011, 2012, 2013 growing period from June to July. For flow direction identification of pollutants (determination of a possible source of contamination), the environmental genotoxic load on different slope exposures and in various altitudes was evaluated. As many studies describe the influence of factors such as altitude or geology subsoil type on the concentrations of certain selected elements in biological matrices or in soil by chemical analysis, this study aimed to verify also not yet analyzed relationship between these factors and pollen abortivity - the overall environmental genotoxicity. A further objective was to establish the accumulation potential of *Pinus mugo* species and differences in the Pb and Cd distribution to various parts of the plant (1-2 years old needles).



Detail of sites 31-47 with indication of control site (45 - yellow point) and sites of vertical transect (32-38 - in set)

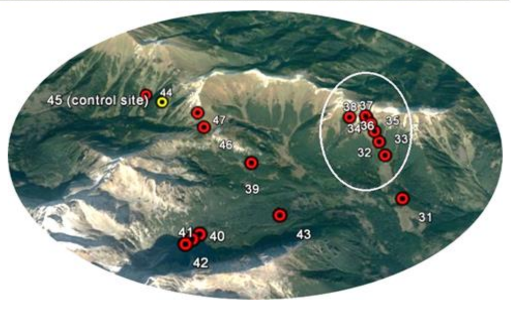


Figure 1. The map of sampling sites: **Lucanska Little Fatra Mts**.: Martinske hole Mt. (Site 1-3); **Krivanska Little Fatra Mts**.: Suchy Mt. (4-7), Krivan Mt. (8-11); **Great Fatra Mts.:** Borisov Mt. (12-15), Krizna Mt. (16-19); **Oravske Beskydy Mts**.: Babia hora Mt. (20-25); **Chocske vrchy Mts.:** Choc Mt. (26-30); **Belian Tatra Mts**.: Bujaci vrch Mt. (31-38), Zadne Medodoly Valley (44, 45, 47); **High Tatra Mts.:** Velke Biele pleso Lake (39), Zelene pleso Lake (40, 41), Zelene pleso Valley (42, 43), Kopske sedlo Seddle (46), Velicka dolina Valley (48-53**); Low Tatra Mts**.: Kralova hola Mt. (54-58); **Western Tatra Mts.:** Brestova Mt. (59-63), Sivy vrch Mt. (64-69). Polygons - three groups of sites to evaluate various slope exposures.

Material

Pinus mugo **Turra**. (2n=24)**: pollen grains and needles** (1-2 years old needles)

- meets all basic criteria for selection of appropriate bioindicator species [1]
- widely distributed in high mountain ecosystems of Western Carpathians
- easily determinable
- quality pollen grains with high sensitivity to air pollutants
- coniferous species

Soil samples

Collection period - growing period from June to July

- **2011** - all 69 sampling sites
- **2012** - only the highest site from each mountain and walley and sites of vertical gradient
- **2013** - only vertical gradient (7 sites in Belian Tatra Mts)

Selection of sampling sites

From 9 mountain ranges

13 mountains

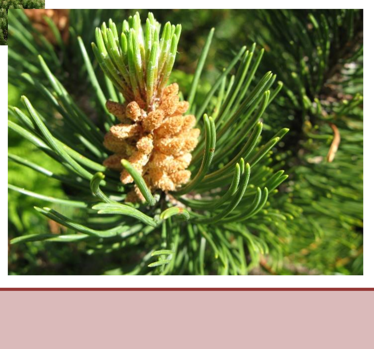
69 sampling sites

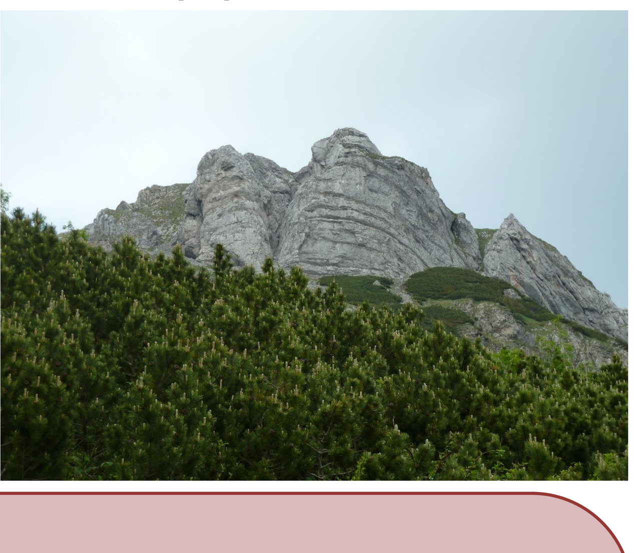
various altitudes: 1,316 - 1,826 m. asl (every 50-100m)

various slope exposures

various geology subsoil

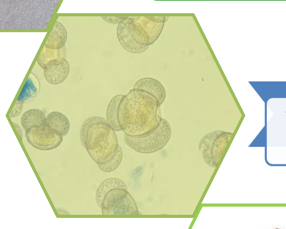
Control site - in Belian Tatra Mts.





Methods

10 randomly chosen branches from 3 individuals → rinsed with distilled water → needles divided according to the age into 1yr-old and 2yr-old needles → dried (68°C/24h) – ground – sieved → analysed as a mixed sample from each age class separately [2, 3] → Chemical analysis **AAS**



Young microstrobili from 10-20 tips of the branches from 5 individuals → fixed in a mixture of ethanol (96%) and acetic acid (3:1) → pollen stained by 0.05% aniline blue in lactophenol [1] → evaluated set - 6000 pollen grains per sample → **Microscopy analysis**

Soil – near the roots of all the individuals from which the needles and pollen were collected at site → Sampling depth: 0-10 cm → mixed sample – dried - oven-dried (70°C/2-4h) - homogenized and sieved → **pH** determination, Chemical analysis - **XRF spectrometry** [4]

The abortion pollen grains were determined on the basis of altered and unformed form, more air sacs, higher size and staining deficiency [5]. Induction factor (IF) was calculated: pollen abortivity at the contaminated site/ pollen abortivity at the control site

Slope exposures

Results

Pinus mugo Turra. showed statistically significant increase of pollen abortivity at least one site from each mountain range compared to the control site, with the exception of the Chocske vrchy Mts. The average induction factors (IF) reflected the strongest increase at the Lucanska Little Fatra Mts., where the genotoxic load was 14.7 times higher in comparison to the control site and the lowest genotoxic effect (average IF=1.51) of air pollution was seen at the Chocske vrchy Mts. (Figure 2). The results of mountain tops pointed to a higher genotoxic load in 2012 than in 2011 on the most mountains, with the exception of Martinske hole Mt., Suchy Mt. and Kopske sedlo Seddle (Figure 3). Pollen abortivity at the control site was 0.15±0.05. The vertical transect (Belian Tatras) showed the gradual increase of pollen abortivity with increasing altitude (Figure 4). A positive correlation between needle Pb content and altitude (Pb1yr: r = 0.68) there was found. The results of this transect did not confirm the correlation between altitude and needle Cd content (Figure 5). The highest genotoxicity was found in the 1. group of mountains on the eastern slopes, in the 2. group on the southern slopes and in the 3. group on the northern slopes (Figure 6). Pb and Cd at all sites and also in the 1. group showed the same results as pollen abortivity - the highest average values on the eastern, northern and western slopes (Figure 7). The highest Pb loads was in the Great Fatra Mts (on Krizna Mt)., Chocske vrchy Mts. and Low Tatra Mts. and the lowest values in the Belian and High Tatra Mts. The mountain ranges the most loaded by Cd were the same by Pb - Chocske vrchy Mts. and Low Tatra Mts. Cd also reached the highest average values on the Krizna Mt. A gradual increasing of Pb content with needle age was observed, but there were no significant differences for Cd (Figure 8). The higher genotoxicity and Pb content in soil and needles was found on limestone subsoil (Sivy vrch Mt.) and higher values of needle Cd were on granite (Brestova Mt.) (Figure 9). The correlation between needle Pb and soil Pb was not found. The positive correlation between soil Pb content and abortivity was found only in the tops of mountains analysis.

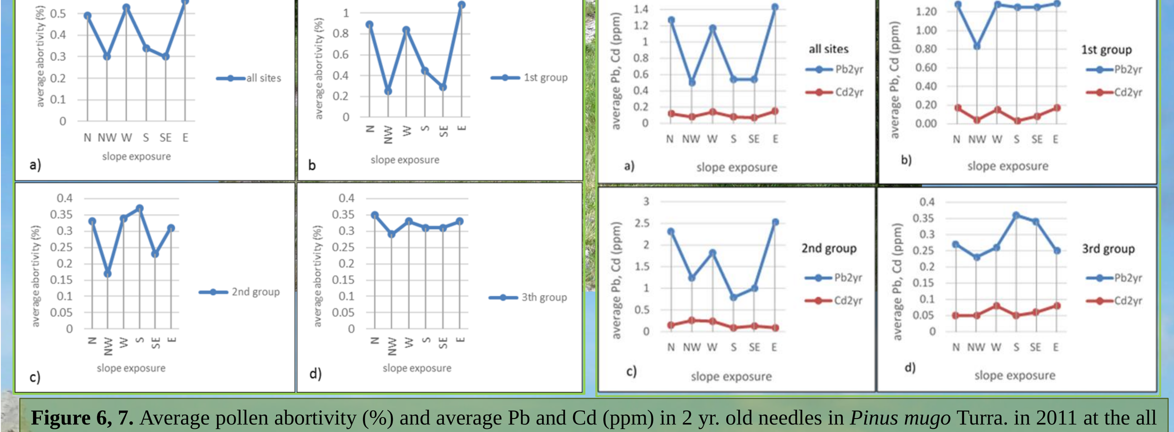


Figure 6, 7. Average pollen abortivity (%) and average Pb and Cd (ppm) in 2 yr. old needles in *Pinus mugo* Turra. in 2011 at the all monitored sites (a), (b) in the **first group** of sites - Little Fatra Mts. (Martinske hole Mt., Suchy Mt., Krivan Mt.), Oravske Beskydy Mts. (Babia hora Mt.) and Chocske vrchy Mts. (Choc Mt.), (c) in the **second group** of sites - Great Fatra Mts. (Borisov Mt., Krizna Mt.) and Low Tatra Mts. (Kralova hola Mt.), (d) in the **third group** of sites - Belian, High and Westhern Tatra Mts. (Bujaci vrch Mt., Velke Biele pleso Lake, Zelene pleso Lake, Zadne Medodoly Walley, Velicka dolina Walley, Brestova Mt., Sivy vrch Mt.) - depending on slope exposure. 95% confidence

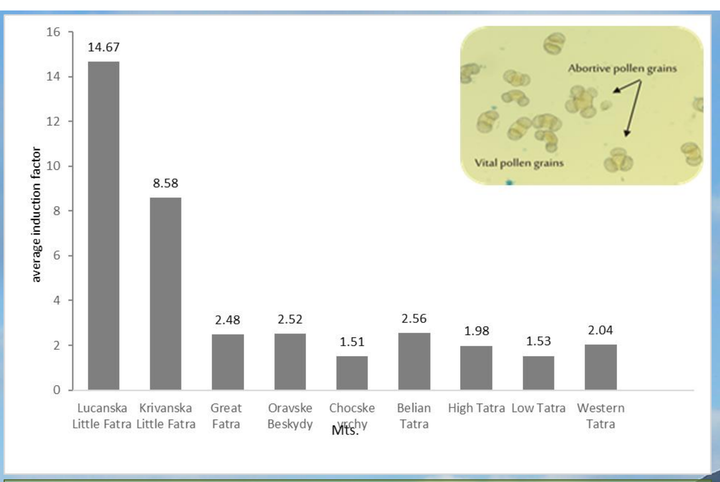


Figure 2. Average induction factors (IF) of evaluated mountain ranges from the all monitored sites in 2011.

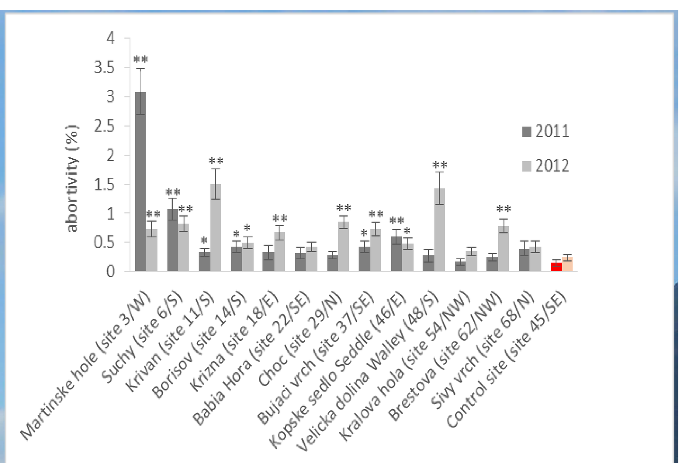
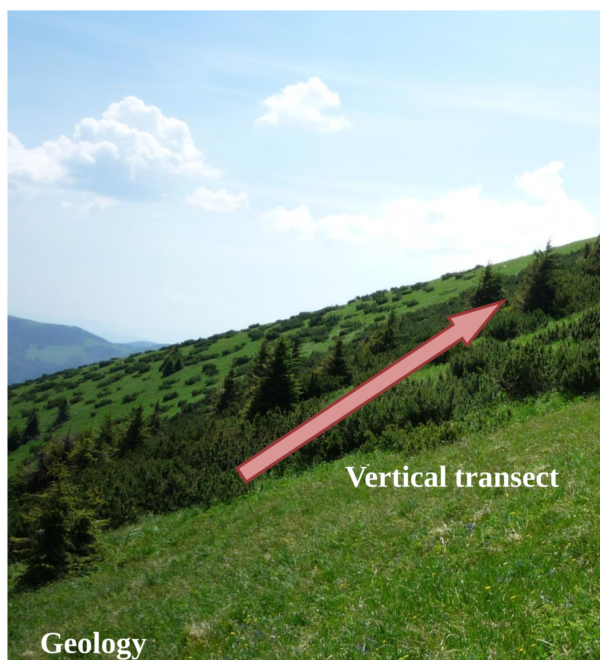


Figure 3. Pollen abortivity (%) measured on the tops of monitored mountains and walleys in 2011 and 2012 and SEM/per 6000 pollen grains. Statistical significance * P≤ 0.05 and ** P≤ 0.01.



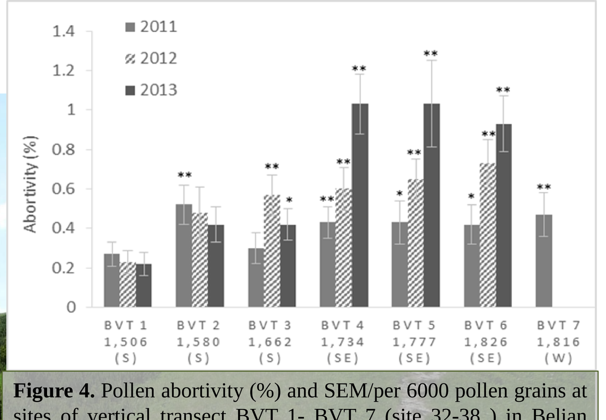


Figure 4. Pollen abortivity (%) and SEM/per 6000 pollen grains at sites of vertical transect BVT 1- BVT 7 (site 32-38) in Belian Tatra Mts. in 2011, 2012 and 2013 with slope exposure. Statistical significance * P≤ 0.05 and ** P≤ 0.01.

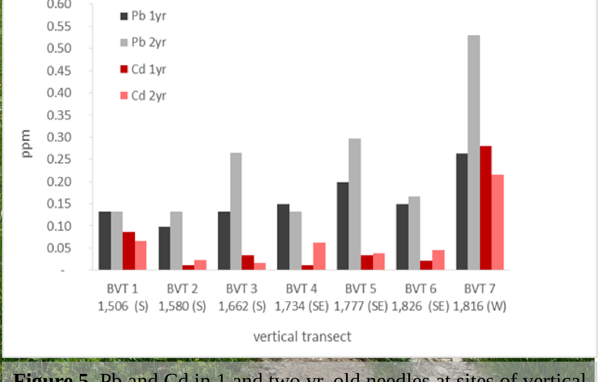


Figure 5. Pb and Cd in 1 and two yr, old needles at sites of vertical transect BVT 1- BVT 2 (site 32-38) in Belian Tatra Mts. at (1,506-1,826 m a.s.l) of the Bujaci vrch Mt. in 2011 with slope exposure.

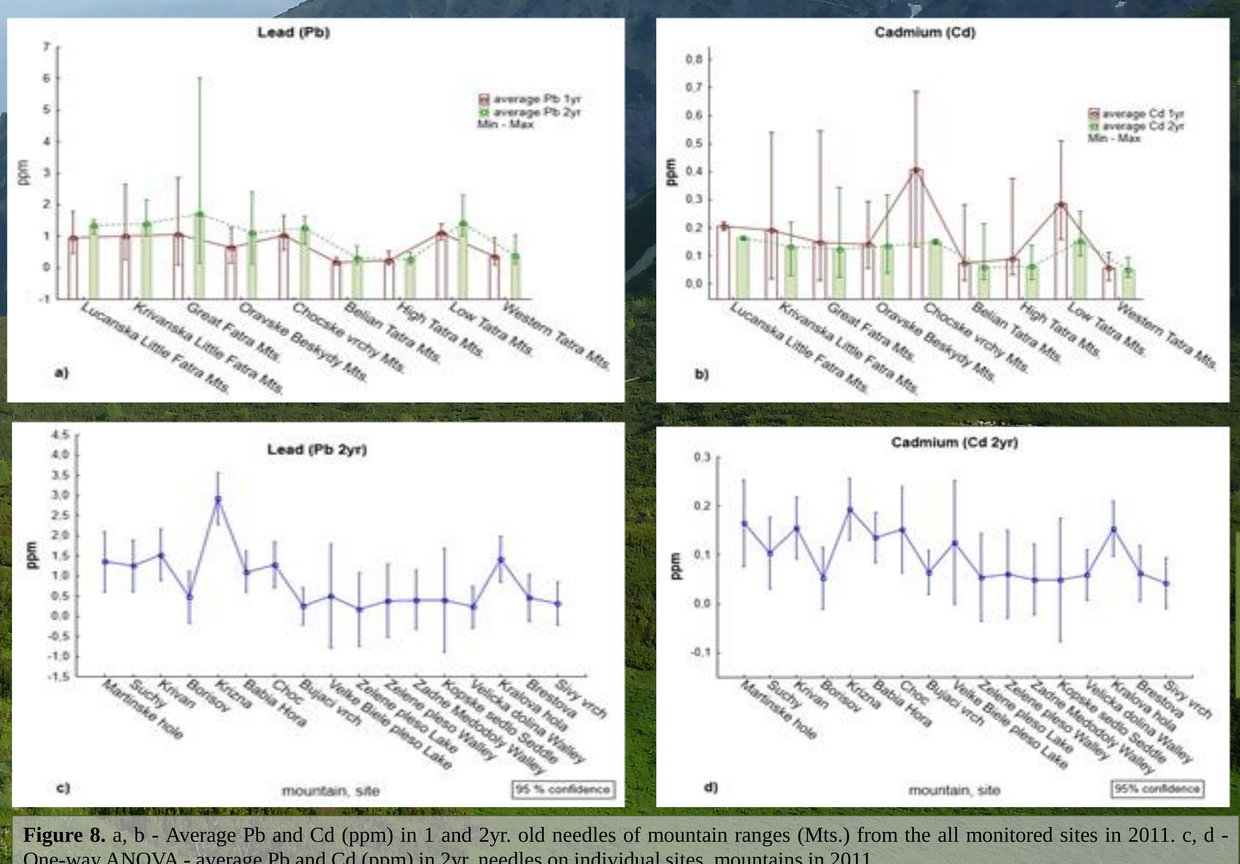


Figure 8. a, b - Average Pb and Cd (ppm) in 1 and 2yr. old needles of mountain ranges (Mts.) from the all monitored sites in 2011. c, d - One-way ANOVA - average Pb and Cd (ppm) in 2yr. needles on individual sites, mountains in 2011.

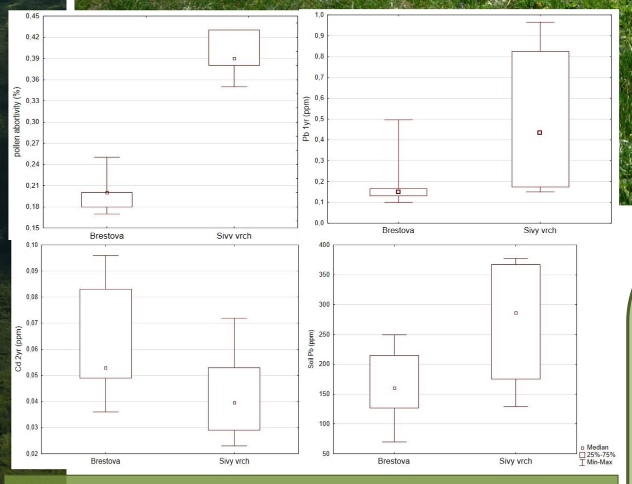


Figure 9. The relationsheep between pollen abortivity (%), concentrations of heavy metals (Pb and Cd) in 1 and 2yr.old needles, soil Pb (ppm) and different geology subsoil at two monitored mountains located close to each other in Western Tatra Mts. - Brestova Mt. (granite): sites 59-63 and Sivy vrch Mt. (limestone): sites 64-69. Samples from 2011.

Conclusion

Our research show that pollen abortion assay allow us to effectively investigate the rate of the genotoxic effect of air mutagenic pollutants also in the mountain and alpine habitates. The test was enough sensitive to indicate the presence of contaminant brought by air currents from far away or even transboundary source of pollution. The test can evaluate a large amount of data (sites, monitor a large area) very quickly, cheaply and without technology that does not reach these sites. Based on the results, then is possible select sites for further more detailed analytical methods. The test evaluates the effect of the total mixture of pollutants on the given areas. The highest genotoxicity on the eastern slopes in the 1. group of mountains was mainly caused by emissions from linear and major industrial sources from territory of Slovakia. Mountains of the Great Fatra Mts. (Krizna Mt. and Borisov Mt.) belonging to the 2. group showed the strongest genotoxic effect on the southern slopes, which were influenced by emissions from the highly industrialized area called the Horna Nitra loaded region with strongly disturbed environment. In the Tatra Mts. region – 3. group industrial areas of Southern Poland (Krakow), Northern Moravia (Ostrava) and Silesia regions represent the major emission sources. Despite a higher values, the pollen abortivity did not exceed the limit of 5% for any of the monitored sites. The highest Pb and Cd concentrations in needles were measured in the Great Fatra Mts. on Krizna Mt. Krizna Mt. was located between the highly-industrialized areas. The western slopes were exposed to emissions from the Turcianska basin, Hornonitrianska basin and Ziarska basin. The eastern slopes were attacted by emissions from the Zvolenska basin and Ziarska basin. The Pb value only on Krizna Mt. exceeded the boundary of normal concentration for plants of 3 ppm **[6].** From 64 analyzed sites, only 4 (Cd1yr.) exceeded the normal values of Cd in plants (0.05-0.5ppm) **[7]** and 6 (Cd1yr.) and 2 (Cd2yr.) sites had higher values than normal threshold value (0.3 ppm) for unpolluted sites **[6].** So it can be stated that the analyzed Slovak mountains are not significantly burdened by these pollutants.

More detailed results will be published in article prepared for journal Ecological Indicators (IF - 4.49)

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