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

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ABSTRACT

This study evaluated the environmental genotoxic load of mountain and alpine habitats of Slovak mountains caused by the total mixture of air pollutants at 69 sites using the phytoindicator *Pinus mugo*. The level of overall genotoxicity was determined by pollen grain abortion assay. This shows that the test is sensitive enough to detect contamination from more distant sources, including long-range transboundary pollutants. It extends the applicability of this test to mountain environments. Soil and plant samples (pollen, one-two year old needles) were collected during a three-year growing period (2011–2013). The study identified source regions of pollutants by assessments at different altitudes and slope exposures. The highest genotoxicity was found in the Little Fatra Mts. on Suchy Mt., with pollen abortivity 3.52 % which represented a 23.5 times higher genotoxic load than at the control site. Samples from mountain peaks showed a higher genotoxicity in 2012 than in 2011 on most mountains. A gradual increase of pollen abortivity and needle Pb content (one year old) with increasing altitude was found in the vertical transect in Belian Tatras. The influence of subsoil type was analysed; higher genotoxicity and Pb content in soil and needles were found on limestone subsoil (Sivy vrch Mt.) and higher values of needle Cd were on granite (Brestova Mt.). The highest Pb and Cd concentrations in needles were measured in the Great Fatra Mts. on Krizna Mt. situated between highly-industrialized areas. The other mountain ranges with the high loading by Pb, Cd were Chocske vrchy Mts. and Low Tatra Mts. The lead content increased with needle age, but the correlation between soil Pb and needle Pb was not confirmed. The positive correlation between soil Pb and abortivity was found only at mountain peaks. The results showed a significant impact of both local industrial and transboundary sources of pollutants. Based on the results, the studied Slovak mountains were not strongly burdened by Pb, Cd and an overall mixture of air pollutants. This methodological approach may contribute to the assessment of urban exposome by comparing the external exposure in urban environment of big cities with background - mountain areas, where the only possible source is long-range transport.

1. Introduction

Heavy metals are the subject of frequent study due to their high concentrations in the environment and significant detrimental effects on all life forms. These pollutants, emitted into the atmosphere, may induce changes in whole ecosystems (Bashmakov et al., 2005). Heavy metals circulate in geochemical and biological cycles without degrading, which results in a gradual accumulation in the environment (Tůma et al., 2005). This poses a serious problem, especially in the immediate vicinity of urban industrial areas (e.g. metallurgy in Katowice-Cracow region, Silesian region in Poland) (Dmuchowski and Bytnerowicz, 1995). Sources are no only local contamination from industrial and urban areas (Calzoni et al., 2007; Sawidis et al., 2011), but also transboundary transport and contamination of high mountains habitats by heavy metals (e.g. Zechmeister, 1995; Šoltés, 1998; Yilmaz and Zengin, 2004; Lee et al., 2005; Kuang et al., 2007, Šoltés and Gregušková, 2012; Šoltés et al., 2014). Because heavy metals can be transported over long distances, the evaluation of their impact in more distant surroundings

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(including mountains) is also important. Deposition of heavy metals is strongly correlated with high levels of precipitation in alpine zones. Increasing concentrations (e.g. Pb, Cd) with rising elevation is caused by a higher heavy metal fallout at higher elevations (Zechmeister, 1995). They are still considered one of the most toxic, globally well-distributed and dangerous environmental pollutants (Ernst et al., 2000; Sinha et al., 2006; Jiang and Liu, 2010). Lead (Pb) and cadmium (Cd) pose a potential risk to human health. They may act as promoters of numerous toxicological effects (mutagenic, carcinogenic) (Palus et al., 2003; Manikantan et al., 2010; Wultsch et al., 2011). Genotoxic effects of lead have been reviewed by García-Lestón et al. (2010). Lead has turbagenic and clastogenic effects. It causes a decrease of mitotic activity and increase of chromosomal abnormalities (Patra et al., 2004; Kumar and Tripathi, 2008).

Plants, due to their high sensitivity and ability to accumulate harmful substances, are used as suitable model organisms to evaluate toxic and genotoxic effects of heavy metals (Patra et al., 2004; Bashmakov et al., 2005; Kumar and Tripathi, 2008; Ghani et al., 2010; Nas and Ali, 2018). Atmospheric heavy metal contamination relates to the decrease of sexual reproduction, pollen viability and frequency of cell division (Izmaiłow and Biskup, 2003; Calzoni et al., 2007; Kormutak et al., 2007; Speranza et al., 2010). Whereas, a huge amount of toxic substances have the same impact on the plants, animals and humans, vascular plants are excellent bioindicators of cytotoxic and mutagenic effects of chemicals in the nature. The organization of the plant chromosome is similar to humans. Pollutants - mutagens cause chromosomal aberrations (Grant, 1978). Plant chromosomes are more sensitive to gaseous emissions and have a wider range of genetic variability than animal indicators (Mejstřík, 1989). Plants are exposed to pollutants, which together contribute to the overall biological effect. The overall toxicity of the environment can be reflected through pollen analysis (pollen grain abortion assay) (Murín, 1987; Mičieta and Murín, 1996a, 1998; Solenská et al., 2006; Mišík et al., 2006, 2007; Gregušková and Mičieta, 2013; Pogányová et al., 2017). Long-term effects of exposure to emissions can be observed by arborescent phytoidicators (Eriksson et al., 1989; Mičieta and Murín, 1996a, 1998; Sawidis et al., 2011). According to Baráková et al. (2018), coniferous species (e.g. *Pinus mugo*) are suitable passive air samplers to assess genotoxic effects of air pollution. *Pinus mugo* is widely distributed in high mountain ecosystems of the Western Carpathians (Solár and Janiga, 2013), easily identifiable, and provides quality pollen grains with high sensitivity to air pollutants.

Trace metal concentrations in plant tissue should be analysed and interpreted in relation to other information such as soil concentrations, root zone and biomass production (Mertens et al., 2005). Easy uptake by roots is caused by an insufficient ability of transport proteins to distinguish ions of these pollutants from elements necessary for plant life (Kumar and Tripathi, 2008). Endoderm such as a barrier holds the vast majority of heavy metals in the root cap, slime and cell walls of rizoderm. Only a portion is transported to the tops of the shoots (Ernst et al., 2000; Patra et al., 2004; Sinha et al., 2006). Transport barriers protect shoots from high concentrations of phytotoxins (Bashmakov et al., 2005). The tolerance to heavy metal toxicity is species-specific, even within the same genus (Meerts and Van Isacker, 1997). The entry pathways to the plants for pollutants are, in addition to the roots, also fissures in the cuticle and open vents. Pine needles have been used as a suitable bioindicators in many studies: *P. sylvestris* L. (Dmuchowski and Bytnerowicz, 1995; Rautio et al., 1998; Yilmaz and Zengin, 2004; Kupcinskiene and Huttunen, 2005), *P. massoniana* Lamb. (Kuang et al., 2007; Sun et al., 2010), *Pinus nigra* Arn. (Sawidis et al., 2011), *Pinus spp.* (Serbula et al., 2013). Ratola et al. (2011, Ratola et al, 2014) described needles of several species of genus Pinus as passive bio-samplers for biomonitoring of airborne organic contaminants (polybrominated diphenyl ethers - PBDEs, organochlorine pesticides - OCPs). Contamination of high mountain areas by air pollutants is still a poorly-studied issue.

The main objectives of this study were **i)** to evaluate the overall genotoxicity of the environment caused by total mixture of all air pollutants by pollen analyses, **ii)** to highlight the applicability of pollen grain abortion assay for pollutant evaluation in mountain environmant, where only pollutants from long-range transport can be expected, **iii)** determine the level of contamination of the mountain and alpine habitats of the Slovak mountains by selected heavy metals - Pb, Cd through the soil and pine needle analyses, **iv)** to evaluate the relationship between pollen abortivity and Pb, Cd concentrations, **v)** to identify the flow direction of the pollutants (i.e., determine a possible sources of contamination) according to evaluation on different slope exposures and various altitudes, **vi)** to evaluate the influence of factors such as altitude and geology subsoil type on the overall environmental genotoxicity (pollen abortivity) and on the Pb, Cd concentrations in the pine needles and in the soil. In the case of pollen abortivity, this relationship was not-yet-analyzed, **vii)** 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 year old needles).

2. Material and methods

2.1. Sampling and analysis

Sampling sites (69) were situated in the mountain and alpine habitats of the Western Carpathians (Fig. 1).

Sampling was conducted on various slope exposures at elevations from 1,316 to 1,826 m a.s.l approximately every 50–100 m. One site in the Belian Tatra Mts (site 45) was chosen as a control site, which is situated in part of the Tatra mountains with the long-term lowest contamination. It was located on the leeward slope (SE), far away from all sources of pollutants. Collection of soil and plant samples was done during the 2011–2013 growing period from June to July. In 2012, only the highest elevation site from each mountain and valley was chosen and seven localities in Belian Tatra Mts. were evaluated to assess the vertical gradient in 2013.

At each site, samples of branches and male strobili with pollen grains of *Pinus mugo* and soil were collected. Each sample consists at least of 10 randomly chosen branches from three individuals. Samples were rinsed with distilled water. Needles were separated from the branches and divided according to the age into 1 and 2 year old needles. All needles were dried at 68 °C for 24 h, ground into a fine powder, sieved and analysed as a mixed sample from each age class separately (Yilmaz and Zengin, 2004; Kuang et al., 2007; Chropeňová et al., 2016). Mineralized samples of needles were analyzed by electrothermal atomic absorption spectrometry (AAS) according to the relevant technical standard (STN EN ISO, 2004). More details are in the Appendix (Text SI 1).

Young microstrobili from 10 to 20 tips of the branches from five individuals were harvested and fixed by a mixture of ethanol (96%) and acetic acid (3:1). Pollen were stained by 0.05% aniline blue in lactophenol (Murín, 1995; Gregušková and Mičieta, 2013; Chropeňová et al., 2016). The aborted pollen grains were identified by altered and unformed form (larger number of air bags), larger size and staining deficiency (Mičieta and Murín, 1996a, 1998). The percentage of pollen abortivity was evaluated (from 6000 pollen grains per sample). For analyses a Nikon YS2 Alphaphot light microscope (400-times magnification) was used. The increase or decrease of genotoxicity of a monitored site in comparison with the control site was expressed by an indicator - induction factor (IF), which reflected the ratio of pollen abortivity at the contaminated vs. the control site (Mišík et. al., 2006).

Soil samples were taken in close proximity to the roots of all individuals from which the needles and pollen were collected, and then a composite sample was prepared. The sampling depth was 0–10 cm. Soils were air dried under ambient temperature and then oven-dried (2–4 h, 70 °C). The samples were subsequently homogenized and sieved (U.S. EPA (U.S. Environmental Protection Agency), 1998). Determination of pH was carried out by using soil pH meter (KS 501). For chemical analysis X-ray fluorescence spectrometry has been used (Clark et al.,

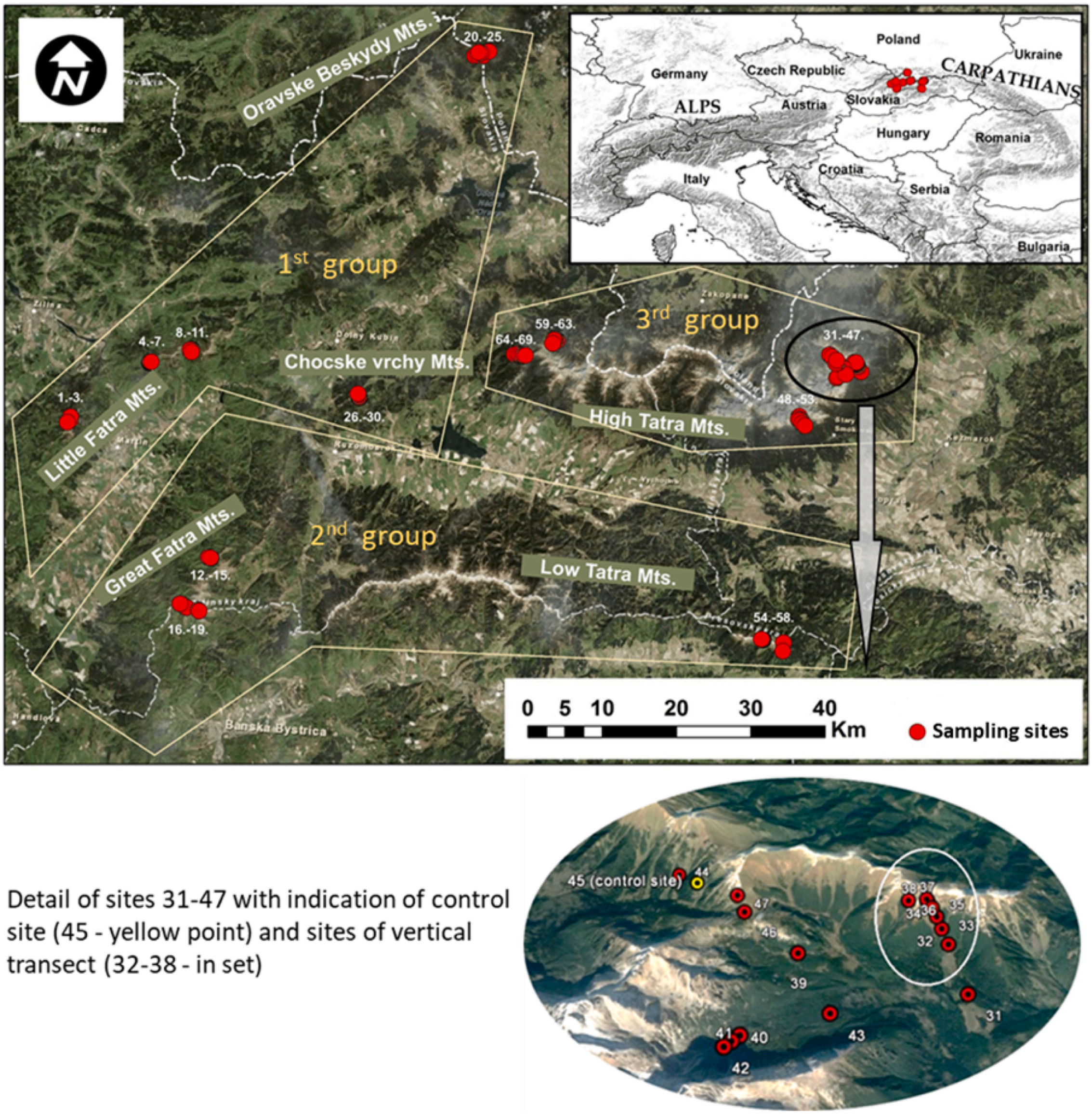

Fig. 1. The sampling sites: **Lucanska Little Fatra Mts.**: Martinske hole Mt. (Site 1–3); **Krivanska Little Fatra Mts.**: Suchy Mt. (4–7), Krivan Mt. (8–11); **Great Fatra Mts.**: Borisov Mt. (12–15), Krizna Mt. (16–19); **Oravske Beskydy Mts.**: Babia hora Mt. (20–25); **Chocske vrchy Mts.**: Choc 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.

1999). More details are in the Appendix (Text SI 2).

2.2. Statistics

Statistically significant differences between pollen abortivity at the control and polluted sites were analyzed by a Student's T-test at 0.05 and 0.01 significance levels. Abortivity on vertical transect and three sampling years was tested by Two-way ANOVA (factors: altitude, year). The correlation between Pb and Cd content in plant and soil samples and pollen abortivity was assessed by using Pearson's correlation coefficient and linear regression analysis. The differences in pollen abortivity, Pb and Cd content between two sites with different geology subsoil were tested with nonparametric discriminant analysis (NDA) and T-test (in the case of Pb, Cd with 0.1 significance level). Two-way ANOVA was used to compare Pb and Cd concentrations (1 yr and 2 yr old needles) between individual mountains. The differences in Pb, Cd content between 1 yr and 2 yr old needles were tested by T-test.

3. Results and discussion

3.1. Pollen abortivity

Fig. 2 shows that the contamination of high mountain habitats by air pollutants damages the genetic material in *Pinus mugo* species. *Pinus mugo* fulfill the all *"Selection criteria for suitable bioindicator species"* for evaluation of pollen abortivity. It produces viable, well-developed pollen grains under normal climatic conditions with pollen abortivity not exceeding 5% (Murín, 1987, 1995). This pollen abortion limit for normal and quality pollen was not exceeded in our research at any sites. The average induction factors (IF) of nine studied mountain ranges are shown in Fig. 2. The highest genotoxicity was found on the eastern slope of Suchy Mt. (site 4) (Krivanska Little Fatra Mts.), with pollen abortivity 23.5 times higher than at the control site, and on the western slope, with induction factor 22 (site 5). Similar high values were measured in Lucanska Little Fatra Mts. on the western slope of Martinske hole Mt.

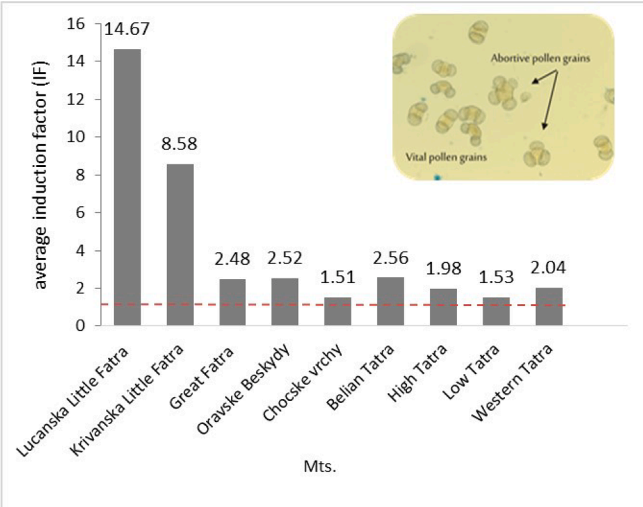

Fig. 2. Average induction factors (IF) of studied mountain ranges from all sites in 2011. Dashed red line: IF at the control site (site 45) = 1. Top right: vital and damaged pollen grains. Evaluation using average pollen abortivity (%) with SEM values is shown in the Appendix (Fig. SI 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(site 3; IF = 20.5) and on the eastern slope (site 1; IF = 15.5). Lower values than at the control site were registered in the High Tatra Mts.: Velicka dolina Valley (sites 49, 53) and in the Oravske Beskydy Mts.: Babia hora Mt (site 24). Pollen abortivity at the control site was 0.15 ± 0.05. Frequencies of pollen abortivity from all sites are summarized in Table SI 1.

3.1.1. *Pollen abortivity vs. Slope exposure*

The evaluation of genotoxicity at various slope exposures indicated the potential source and dispersion direction of the highest pollutant concentrations. All sites were divided into three groups (Fig. 3) according to the possible impact of stationary and line pollutant sources. According to NEIS (Slovak National Emission Information System) (2016) (Slovak National Emission Information System), the largest stationary sources of basic air pollutants affecting the monitored area were heating plants in the cities of Zilina and Martin, production of ferro-alloys in Istebne, pulp mill in Ruzomberok, aluminum plant in Ziar nad Hronom and power plants in Zemianske Kostolany. *Pinus mugo* was used for bioindication of contamination near the pulp mill in Zilina with pollen abortivity 1.3 ± 0.16 (Chropeňová et al., 2016), in the vicinity of aluminium plant in Ziar nad Hronom (4.4 ± 1.3) and near the Botanical Garden of Bratislava (2.4 ± 0.2) (Mičieta and Murín, 1998). In our study, the values ranged from 0.1 to 3.52 %.

The prevailing north and northwest winds caused the overall highest genotoxicity on the western slopes at all sites and at the 1st group of sites (Fig. 3). This highest genotoxic load in the 1st group of mountains was mainly caused by transboundary pollutants (form Northern Moravia, Southern Poland) and emissions from urban and industrial stationary sources in individual districts. The values of basic air pollutants measured in 2011 (Table 1) pointed to a higher burden of the Zilina region, especially in the districts of Martin and Zilina (NEIS (Slovak National Emission Information System), 2016). The highest abortivity values on this exposure were found on Martinske hole Mt. (site 3; 3.08 ± 0.39; IF = 20.05) and Suchy Mt. (site 5; 3.30 ± 0.57; IF = 22.0). These mountains of Little Fatra Mts represent the first capture point, a natural barrier to pollutants coming from the west (from Zilina district and Northern Moravia). The environmental genotoxicity in Zilina city caused by industrial and traffic pollutants was described in several studies (Solenská et al., 2006; Chropeňová et al., 2016; Mičieta, 2021). The second highest genotoxic load on the eastern slopes was affected by linear and industrial pollutants from district of Martin which caused the highest abortivity from this study on Suchy Mt (site 4; 3.52 ± 0.09; IF = 23.05). Gregušková and Mičieta (2013) reported the high genotoxic effect of these pollutants in Martin city (between Little and Great Fatra Mts.).

Mountains of the Great Fatra Mts. (Krizna Mt. and Borisov Mt.) from the 2nd group showed the highest genotoxicity on the southern slopes affected by emissions from the highly industrialized area called the Horna Nitra loaded region in Central Slovakia (cities Zemianske Kostolany, Novaky) with strongly disturbed environment (MŽP and SAŽP, 2016a). According to the Environmental Regionalization of Slovakia (MŽP and SAŽP, 2016b), the other affecting industrial cities Ziar nad Hronom, Banska Bystrica, Zvolen belong to the zones with greatly disturbed and disturbed environments (Fig. S1 2). A pollen abortion

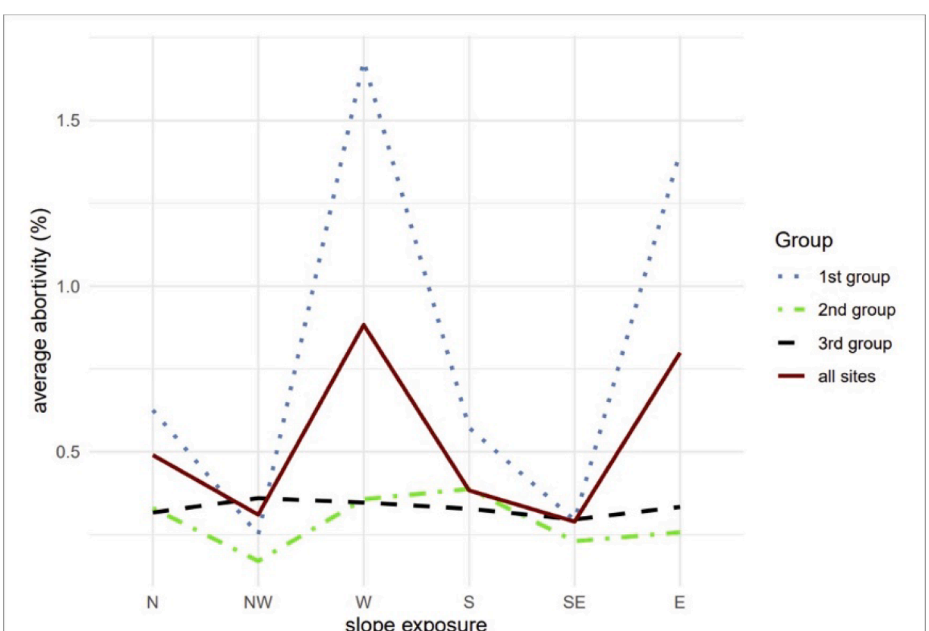

Fig. 3. Average pollen abortivity (%) in *Pinus mugo* in 2011 at **all sites** (red solid line); in the **1st group** of sites (blue dotted line): Little Fatra Mts. (Martinske hole Mt., Suchy Mt., Krivan Mt.), Oravske Beskydy Mts. (Babia hora Mt.), Chocske vrchy Mts. (Choc Mt.); in the **2nd group** of sites (green dotted-dashed line): Great Fatra Mts. (Borisov Mt., Krizna Mt.), Low Tatra Mts. (Kralova hola Mt.); in the **3rd group** of sites (black dashed line): Belian, High and Westhern Tatra Mts. (Bujaci vrch Mt., Velke Biele pleso Lake, Zelene pleso Lake, Zadne Medodoly Valley, Velicka dolina Valley, Brestova Mt., Sivy vrch Mt.) - depending on slope exposure. (95% confidence). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Emissions from stationary sources in Slovak regions in 2011, 2012 and 2013 for Presov region (in tonnes) (NEIS, Slovak National Emission Information System, 2016).

Region	District	Year	PM	SO_2	NO_X	CO	Cd	Pb	Our sites (Mt)
Zilina	Zilina	2011	122.6	625.3	613.9	1,700	0.002	0.016	Suchy, Krivan
		2012	132.2	509.5	548.0	200.9	0.003	–	
	Martin	2011	30.3	748.1	321.0	154.6	–	0.002	Martinske hole, Borisov, Krizna
		2012	26.6	874.1	333.4	127.6	–	0.001	
	Ruzomberok	2011	260.5	218.9	1,392	488.1	0.027	0.027	Choc
		2012	230.8	146.0	1,256	496.3	–	–	
	Namestovo	2011	31.2	29.9	25.4	79.0	–	–	Babia hora
		2012	28.4	27.0	24.9	77.3	–	–	
	Tvrdosin	2011	13.2	0.8	24.2	16.6	–	–	Brestova, Sivy vrch
		2012	11.1	0.6	23.4	16.9	–	–	
Presov	Poprad	2011	27.1	1.5	106.3	73.3	–	–	Tatra Mts.
		2012	22.5	1.4	99.5	120.8	–	–	
		2013	19.1	1.4	93.6	143.7	–	–	
Banska Bysrtica	Brezno	2011	66.0	33.9	133.4	450.7	–	–	Kralova hola
		2012	71.0	45.3	140.1	449.6	–	–	

assay with 17 wild species near the aluminum plant in Ziar nad Hronom identified the environmental genotoxic burden with an average annual abortion rate of 4.3% (2011) and 4% (2012) (Pogányová et al., 2017). Mičieta (2021) evaluated the long - term effect of traffic emissions by pollen analysis in Ziar nad Hronom. In our study, Krizna Mt., as the first mountain exposed to these emissions, showed the higher pollen abortivity (0.47 ± 0.09; IF = 3.1) than Borisov Mt. (0.43 ± 0.10; IF = 2.9).

In the Tatra Mts. – 3rd group, industrial areas of Southern Poland (Krakow), Northern Moravia (Ostrava) and Silesia regions represent the major emission sources located at a distance of 150–200 km from the monitored sites (Rak et al., 1982; Šoltés and Gregušková, 2012). The dynamic changes in the air pollution of the High Tatras (over the last 100 years) were evaluated by pollen analysis using herbarium items of *Calluna vulgaris*. High abortivity there could be caused by pollution from heavy industrial areas around Polish coal mines (Ciezsyn, Katowice) (Mičieta and Murín, 1996b, 1999). Our results confirmed the highest abortivity values on the northwestern and western slopes. More details about individual mountains are in the Appendix (Text SI 3).

To eliminate impact of road transport as the most significant source of Pb in Europe (Von Storch et al., 2003), the highest site from each mountain and valley was chosen (above 1,450 m) and evaluated in 2011 and 2012. The results (Fig. 4, Table SI 2, 3) pointed to a higher genotoxic load in 2012 on most mountains. Lower values of basic air pollutants in 2012 in the districts of Zilina (SO_2, NO_X, CO), Martin (CO, PM), Ruzomberok and Poprad (SO_2, NO_X, PM) (Table 1) contributed to a lower genotoxicity on Martinske hole Mt, Suchy Mt and Kopske sedlo Saddle. Despite that, Martinske hole Mt. and Suchy Mt. were the most burdened sites, where a statistically significant increase of pollen abortivity was found in comparison to the control site in both years (Table SI 3). On the other hand, Kralova hola Mt. did not show a statistically significant increase during monitored years. A large difference in the pollen abortivity between these years was found in the Velicka dolina Valley, where the higher genotoxic load in 2012 was partly due to an increase of CO emissions in the Poprad district (Table 1). The impact of the all air pollutants on this site caused pollen abortivity of 0.28 ± 0.11 (IF = 1.9) in 2011 and 1.43 ± 0.27 (IF = 6.0) in 2012. Despite the

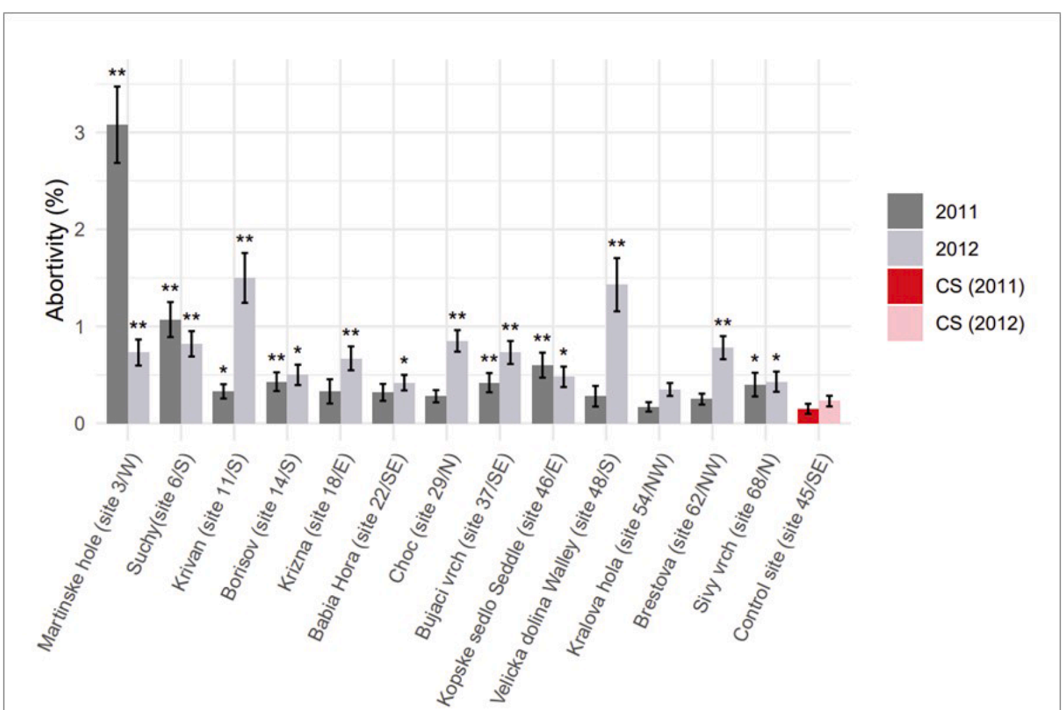

Fig. 4. Pollen abortivity (%) measured on the tops of mountains and valleys (site/slope exposures) in 2011, 2012 and SEM/per 6000 pollen grains in *Pinus mugo*. Control site (CS): Zadne Medodoly Valley (site 45). T-test with statistical significance * $p \leq 0.05$ and ** $p \leq 0.01$ (p - values in Table SI 3).

gradual decline in basic air pollutants in most districts (Table 1), the results pointed to an increased environmental genotoxic burden in 2012. It follows that many other pollutants were involved in the overall effect (e.g. groups of organic pollutants). Chropeňová et al. (2016) confirmed the presence of organic pollutants and heavy metal genotoxicity in alpine ecosystems of the Slovak mountains. Baráková et al. (2018) found a significant correlation between genotoxicity and levels of persistant organic pollutant (PCBs, HCHs, DDTs, HCBs) contamination. Both studies were based on the genotoxic potential of extracts of *Pinus mugo* from Slovak mountains.

3.1.2. Pollen abortivity vs. Altitude

The negative correlation between altitude and pollen abortivity in the 1st ($r = -0.61$, $p = 0.002$) and 2nd group of sites ($r = -0.67$, $p = 0.011$) showed the greater impact of local sources of pollution (traffic, industry) on the localities of Little Fatra Mts., Great Fatra Mts., Low Tatra Mts., Chocske vrchy Mts. and Oravske Beskydy Mts. To assess the vertical gradient, it was necessary to exclude the impact of these sources, which caused high pollutant values at low altitudes (Lee et al., 2005). The vertical transect of selected sites in Belian Tatras (Fig. 5, Table SI 4, 5) indicated a gradual increase in pollen abortivity with increasing altitude ($p < 0.001$). The highest genotoxicity with a high statistically-significant increase in pollen abortivity compared to the control site was observed at the second lowest sampling site (BVT2, site 33; 1,580 m a.s.l; 0.52 ± 0.10; IF = 3.5) in 2011. There it was caused by direct exposure to the air mass flowing from the west. This air flow was routed directly to the monitored site by the relief shape. This result was probably due to the prevailing western winds in 2011. In 2012 and 2013, the highest plant responses to genotoxic pollutants were observed at the highest altitudes. The highest sites were more strongly impacted by transboundary pollutants from southern Poland. The relationship between increasing distance from the emission source and pollen abortivity has been reported in several studies (Mišík et al., 2006; Carneiro et al., 2011; Gregušková and Mičieta, 2013), but the aspect of increasing altitude was not well-documented. Chropeňová et al. (2016) studied this relationship on Sivy Vrch Mt. in the Western Tatras, where pollen abortivity ranged from 0.72 ± 0.11 to 1.25 ± 0.17 (1,515–1,756 m a.s.l). In our study, lower abortivity values (0.22 ± 0.06–0.93 ± 0.14) were due to the lower genotoxic load in the Belian Tatra Mts. compared to Western Tatra Mts. at similar altitudes in 2013. The lowest genotoxic load was found at the first altitudinal point at 1,506 m a.s.l. during all monitored years.

3.2. Pollen abortivity and heavy metals

In our study, a positive correlation between soil Pb and abortivity ($r = 0.67$) was found at mountain peaks in 2012 (Fig. 6). The regression analysis also confirmed the significance ($p = 0.009$). The coefficient of determination ($R^2 = 0.44$) indicated that some other factors also affected pollen abortivity (e.g. other air pollutants, UV radiation, precipitation). Chropeňová et al. (2016) demonstrated that organic pollutants (DDTs, HCHs) had high concentrations on the same mountains as were evaluated in this study (Martinske hole Mt., Suchy Mt., Krizna Mt. and Krivan Mt.). Pogányová et al. (2017) pointed out the possible influence of dilution effect, which resulted in lower abortivity during high precipitation. Pollen grains are very sensitive to genetic changes and also to nonmutagenic extreme environmental factors (temperature, dryness, nutrition deficiences) (Murín, 1987). Temperature and relative humidity affect spontaneous, as well as pollution-induced, mutations. An increase of frequency of Tradescantia micronucleus in urban areas was mainly caused by low temperature, relative humidity and rainfall (Savóia et al., 2009). Mountain ecosystems generally have higher relative humidity, lower temperatures and more precipitation than urban areas. Klumpp et al. (2004) also evaluated the influence of climatic factors on mutations in pollen mother cells of Tradescantia species. The processes of meiosis and pollen development are very sensitive to cold. They found a high increase in spontaneous mutations at low temperatures. The high relative humidity caused lower uptake of pollutants due to the reduction of transpiration, closing of stomata and thus lower pollution-induced mutations. In contrast, acid rain increased foliar

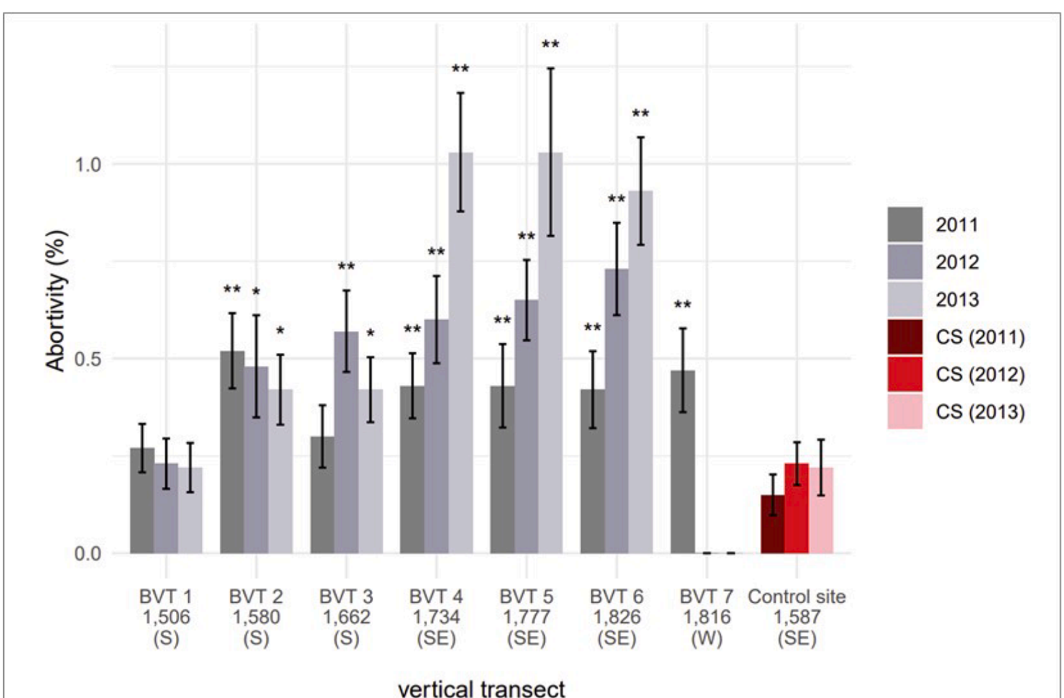

Fig. 5. Pollen abortivity (%) and SEM/per 6000 pollen grains in *Pinus mugo* at sites of vertical transect BVT1 - BVT7 (site 32–38) in Belian Tatra Mts. at gradually increasing altitude (1,506–1,826 m a.s.l) of the Bujaci vrch Mt. in 2011, 2012, 2013 with slope exposure. T-test with statistical significance * $p \leq 0.05$ and ** $p \leq 0.01$ (p - values in Table SI 5). Two-way ANOVA (at 6 sites excluding BVT 7): factors – altitude ($p < 0.001$) and year ($p < 0.001$) were significant.

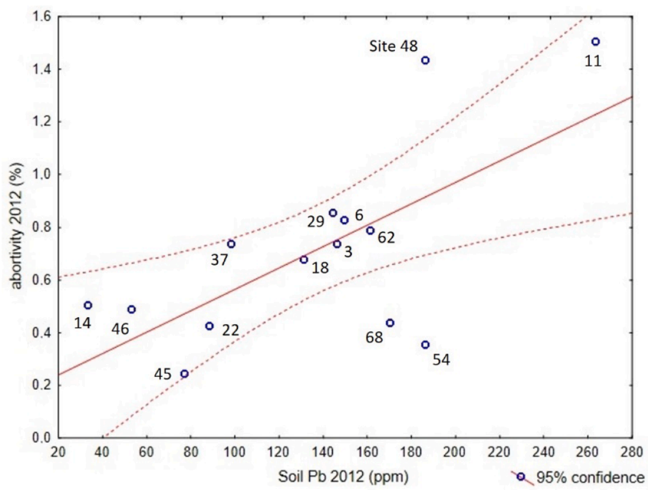

Fig. 6. Relationship between concentration of soil Pb (ppm) and pollen abortivity at sites on mountain peak (2012): Martinske hole Mt. (site 3), Suchy Mt. (6), Krivan Mt. (11), Borisov Mt. (14), Krizna Mt. (18), Babia hora Mt. (22), Choc Mt. (29), Bujaci vrch Mt. (37), Kopske sedlo Saddle (46), Velicka dolina Walley (48), Kralova hola Mt. (54), Brestova Mt. (62), Sivy vrch Mt. (68), Control site (45). p-value: 0.009; r = 0.67.

penetration of particulates with heavy metals as result of stomata deformation, erosion of epistomatic waxes, alteration of the ultrastructure and cuticle thickness (Turunen and Huttunen, 1991; Smidt et al., 1996; Kupcinskiene and Huttunen, 2005). Pollen viability of *Pinus mugo* was tested by Kormutak et al. (2007) in the High Tatras. Sterile pollen (21%) was ascribed to the detrimental climatic conditions for microsporogenesis in high altitudes. Kumar and Tripathi (2008) recorded an increase of chromosomal abnormalities (pollen sterility) with the lead nitrate concentration in the pollen mother cells of grass peas. The relationship between pollen abortivity and Pb in *Rosa rugosa* leaves (Calzoni et al., 2007) and in pine needles (Chropeňová et al., 2016) was documented. In this study, only a weak correlation between needle Pb (1 yr) and pollen abortivity was found for analysis of mountain peaks in 2011 (r = 0.55, p = 0.038) (Fig. SI 3). The hypothesis of correlation between Cd content and abortivity there was not confirmed. Izmaiłow and Biskup (2003) found decreased fertility of *Echium vulgare* at sites heavily contaminated with heavy metals (Pb, Cd) in Silesia, Poland (35–90% degenerated mature pollen). Knasmüller et al. (1998) confirmed the heavy metal genotoxic effects by plant bioassay, Tradescantia micronucleus test. Mišík et al. (2006) found the same rank order of genotoxic effects of different emissions sources in pollen abortion assay as in Tradescantia MN test. Virtually identical results of the pollen abortion assay and EDXRF spectrometry of tree bark (including Pb) for biomonitoring of air pollution by traffic emissions were described by Carneiro et al. (2011). The influence of heavy industry (heavy metals,

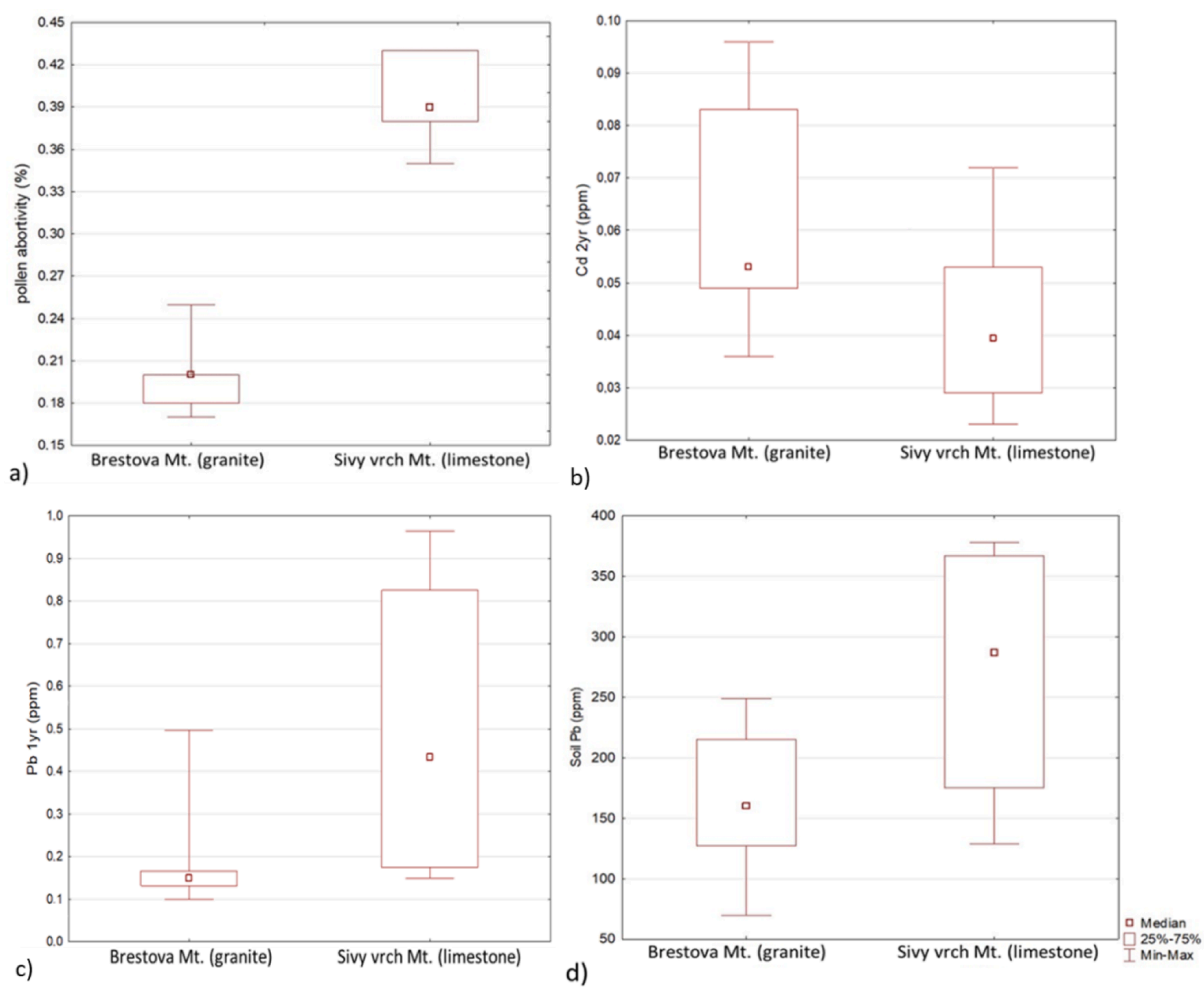

Fig. 7. Comparison of different geology subsoils (granite - Brestova Mt., limestone - Sivy vrch Mt.) in relation to **a)** pollen abortivity (%, T-test: p < 0,001), **b)** needle Cd (2 yr old), **c)** needle Pb (1 yr old.) concentrations and **d)** soil Pb content (ppm). This two mountains located close to each other in Western Tatra Mts. - Brestova Mt.: sites 59–63 and Sivy vrch Mt.: sites 64–69, (in 2011). T- test (0.1 significance level) – in the case of Cd 2 yr (p = 0.07), Pb 1 yr (p = 0.052) and soil Pb (p = 0.045).

organic compounds - PCB, PAH) on pollen abortivity in *Pinus* species was confirmed (Mičieta and Murín, 1996a, 1998).

3.2.1. Pollen abortivity and heavy metals vs. Subsoil geology

The influence of subsoil geology and soil pH on pollen abortivity, soil Pb content and heavy metal (Pb, Cd) accumulation in *Pinus mugo* needles is summarized in Fig. 7. Higher genotoxicity was observed on Sivy vrch Mt. (limestone subsoil) than on nearby Brestova Mt. (granite subsoil) although samples were collected under the same conditions (time, altitudes, slope exposures) (Fig. 7a). A statistically significant difference ($p < 0.001$) in pollen abortivity between these two mountains was confirmed by T-test. A positive Pearson's correlation between pollen abortivity and soil pH was found ($r = 0.86$, $p = 0.0006$).

Soil parameters such as soil reaction, organic matter, mineral composition, oxides content and activity of soil microorganisms affect heavy metal mobility in the soil (Yong et al., 1992; Vollmannová et al., 2004; Makovníková et al., 2006). Generally, cadmium is characterized by high mobility in soils. The bioavailability of metals depends mainly

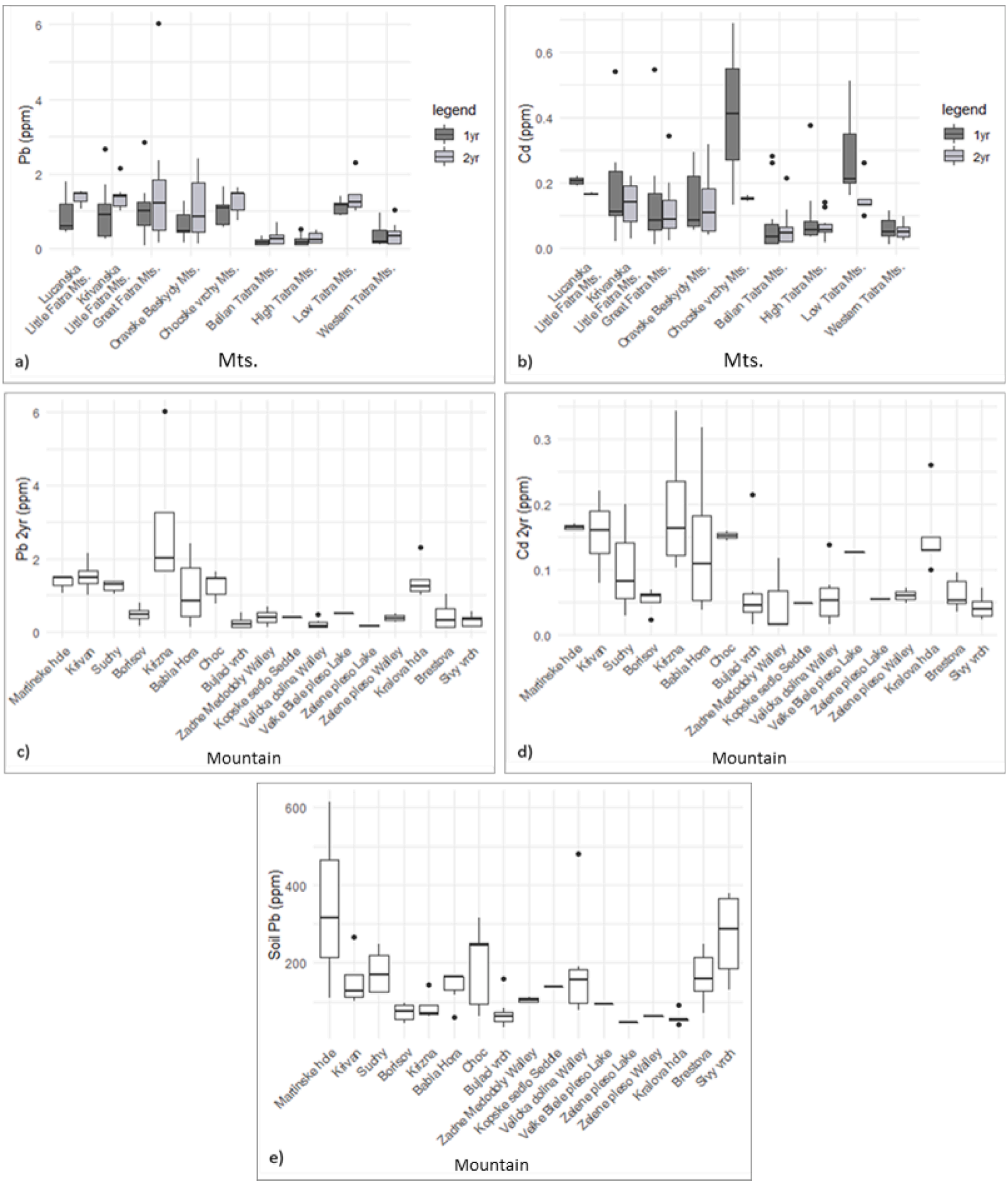

Fig. 8. a, b – Concentration of Pb and Cd in one and two-year-old needles (1 yr and 2 yr) for individual mountain ranges (Mts.) from all monitored sites (Median, 25–75 percentiles, min–max values). Cadmium analyses exclude sites with extremely high values (Martinske hole Mt.- site 1; Choc Mt. - site 26; 28; 30; Suchy vrch Mt.- site 5). Two-way ANOVA - average Pb (Mts.: $p < 0.001$; age: $p = 0.022$) and Cd (Mts.: $p < 0.001$; age: $p = 0.04$). **c, d, e** - One-way ANOVA - average Pb ($p < 0.001$), Cd ($p = 0.009$) in 2 yr needles and soil Pb ($p = 0.001$) on individual mountains; in 2011. (95% confidence).

on the soil pH (Alloway, 1990). As in our study, Tóth et al. (2006) showed that cadmium was more available to plants on acidic soils. We found higher cadmium values in needles (Cd2yr) on Brestova Mt. (granite), where the average pH was 4.28 (Fig. 7b). A negative Pearson's correlation ($r = -0.71$, $p = 0.015$) between Cd2yr and soil pH was measured. According to Yong et al. (1992), Cd mobility and bioavailability were highest on soils with pH 4.5–5.5. Cadmium was less mobile and tended to precipitate on the surface of clay minerals in alkaline soils, while lead showed high binding to clay minerals on acid soils. This study also showed lower lead values on granite subsoil. Higher lead concentrations in needles (Pb1yr) and soil were on limestone (Sivy vrch Mt.) with an average pH 6.23 (Fig. 7c, d). Generally, lead is less mobile with a high affinity to form complex compounds. Ernst et al. (2000) found that low soil pH (3.9) increased Pb and Cd mobility and resulted in higher uptake. In our study, Pb2yr evaluation was influenced by the high value caused by the very low soil pH (3.8) at one of the sites on Brestova Mt. (site 60). For this reason the difference between subsoils was not significant.

3.3. Heavy metals

The main sources of lead and cadmium were local industry, traffic and long-range transboundary transport. The results of chemical analyses of pine needles and soil determination of Pb and Cd are summarized in Table SI 6. The average Pb in one-year-old needles (Pb1yr) was 0.62 ppm and in two-year-old needles (Pb2yr) was 0.86 ppm. Soil lead concentration had an average value of 144.9 ppm (33–614 ppm). The average needle Cd concentrations were 0.13 ppm (Cd1yr) and 0.096 ppm (Cd2yr). The results showed a lower degree of lead contamination of the Slovak mountains compared to a Turkish mountain, where the average Pb in *Pinus sylvestris* needles were 19.01 (1 yr) and 13.05 ppm (2 yr) in 2000 (Yilmaz and Zengin, 2004). A similar Pb values as in Slovak mountains was observed on Xiqiao Mountain in China (average Pb1yr = 0.12 and Pb2yr = 1.39 ppm) (Kuang et al., 2007).

The highest lead loads were found in the Great Fatra Mts., Chocske vrchy Mts. and Low Tatra Mts. The hypothesis of the lowest Pb and Cd values in the Belian Tatra Mts. was confirmed. The same mountain ranges had the highest Pb and Cd contamination - Chocske vrchy Mts. and Low Tatra Mts. (Fig. 8a,b).

The highest needle Pb concentrations were measured in the Great Fatra Mts. on Krizna Mt. at site 17 (Pb1yr = 2.85 ppm; W slope) and at site 18 (Pb2yr = 6.03 ppm; E slope). Krizna Mt. was located between highly-industrialized areas. Its western slope was exposed to emissions from Turcianska, Hornonitrianska and Ziarska basins. The eastern slope was affected by emissions from Zvolenska and Ziarska basins. At only one site (17), the lead exceeded the boundary of normal concentration for plants (3 ppm) reported by Allen et al. (1974) but it was below the amended boundary of 5–10 ppm according to Kabata-Pendias and Pendias (2001). The average needle Pb2yr on Krizna Mt. was 4.25 times higher compared to the control site. Values close to 3 ppm were also found on Krivan Mt. (site 9), Babia hora Mt. (site 23), Kralova hola Mt. (site 56) and Choc Mt. (site 26). Fig. 8c,d,e show the comparison of Pb contamination of individual mountains.

Cadmium, similarly to lead, had the highest average values on Krizna Mt., Kralova hola Mt. and Choc Mt. (Fig. 8d). The highest needle Cd concentrations were on Choc Mt. at site 29 (Cd1yr = 0.69 ppm; N slope) and on Krizna Mt. at site 16 (Cd2yr = 0.34 ppm; W slope). Choc Mt. had a 69 times higher value of needle Cd and Krizna Mt. had 19.5 times in comparison to the control site. From 64 analyzed sites, only 4 (Cd1yr) exceeded the normal values of Cd in plants (0.05–0.5 ppm) determined by Kabata-Pendias and Pendias (2001) and 6 (Cd1yr) and 2 (Cd2yr) sites had higher values than normal threshold value (0.3 ppm) for unpolluted sites according to Allen et al. (1974). This indicates that the studied Slovak mountains are not significantly burdened by cadmium.

3.3.1. Uptake and accumulation

An increase of Pb concentration with needle age was observed (Fig. 8a) (one-sided T-test, $p = 0.031$). It confirmed a gradual accumulation of Pb in plant tissues. According to Wyttenbach et al. (1996), this relationship was mainly caused by foliar uptake. This positive correlation was also described by Sun et al. (2010) and Kuang et al. (2007). Total amount of pollutants, relative humidity and wet deposition could significantly affect the capacity to absorb metals (Lee et al., 2005). Metal availability to plants is highly dependent on the species (Mertens et al., 2005). Heavy metal uptake and effectiveness are determined by the structure of pine leaves and chemical properties of pollutants (Sun et al., 2010). Because needles have relatively impermeabile thick smooth waxy cuticles, sunken stomata represent the main uptake pathway (Sawidis et al., 2011). The erosion of pine needle surface wax caused by exposure to industrial pollutants was described by Kupcinskiene and Huttunen (2005). The increase in the tubular wax-covered needle surface was found as emissions decreased. Yilmaz and Zengin (2004) found higher Pb values in 1 yr than in 2 yr-old needles. Our study did not find significant differences for Cd in relation to needle age (two-sided T-test, $p = 0.069$) (Fig. 8b), similarily to Kuang et al. (2007).

A correlation was not found between needle Pb and soil Pb. Therefore, the amount of needle Pb was mainly caused by uptake from the atmosphere and did not depend significantly on the translocation from soil to leaves. Calzoni et al. (2007) and Serbula et al. (2013) found a similar result. Low extraction coefficients (ratio of needle Pb and soil Pb) with values 0.00035–0.008 showed low ability of *Pinus mugo* to accumulate Pb from soil. The criterium for hyperaccumulator, an extraction coefficient greater than one (Chen et al., 2004), was not achieved at any site. The same results were reported in the study of Ernst et al. (2000), where only 10% of Pb was accumulated in the above-ground biomass. Pb was stored mainly in the roots. Ernst et al. (2000) found a linear increase of Pb in the roots with soil Pb. The amount of metal absorbed from soil and through the leaves is sometimes difficult to distinguish. Trees, as long-lived organisms, reflect cumulative effects of environmental pollution from both soil and atmosphere (Dmuchowski and Bytnerowicz, 1995; Sawidis et al., 2011). Serbula et al. (2013) compared the accumulation ability of *Pinus* spp. and *Tilia* spp. The results showed that pine branches and needles contained more Pb than the same parts of *Tilia* spp.

3.3.2. Heavy metals vs. Slope exposure

Spatial distribution of Pb emissions in Europe was described in several studies (e.g. Dunlap et al., 1999; Von Storch et al., 2003; Pacyna et al., 2009). In our study, the average lead and cadmium concentrations at various slope exposures for all sites and for three separate groups of sites are shown in Fig. 9. The concentrations of Pb (Fig. 9a) and Cd (Fig. 9b) at all sites and in the 1st group showed the same results as pollen abortivity - the highest average values were on the eastern, northern and western slopes. All mountains from the 1st group had eastern slopes burdened by Pb from local industrial sources, traffic and heating, with the exception of Babia hora Mt. There, the higher Pb values found on the eastern (site 23: 2.42 ppm), northern and northwestern slopes, suggested Poland as the source region of higher concentrations. This transboundary Pb together with local contamination (e.g. nearby ferroalloy production) also caused a high load on the northern slopes. Dmuchowski and Bytnerowicz (1995) monitored the distribution and environmental burden of heavy metals in Poland by pine needle analyses. High pollution (Pb, Cd) was found in the Katowice-Cracow industrial region. Toxic values were found in the most stressed zones, for Pb > 30 ppm (Upper Silesian region) and for Cd > 5 ppm. The western slopes were exposed to Pb from Zilina district further enriched with transboundary pollutants from northern Moravia. Martinske hole Mt., as the first mountain receiving pollution from the west, showed the highest Pb of all western slopes of this group (site 3: 1.52 ppm).

In the 2nd group, the highest average Pb2yr on the eastern slope was due to the high value on the Krizna Mt. (site 18: 6.03 ppm) caused by

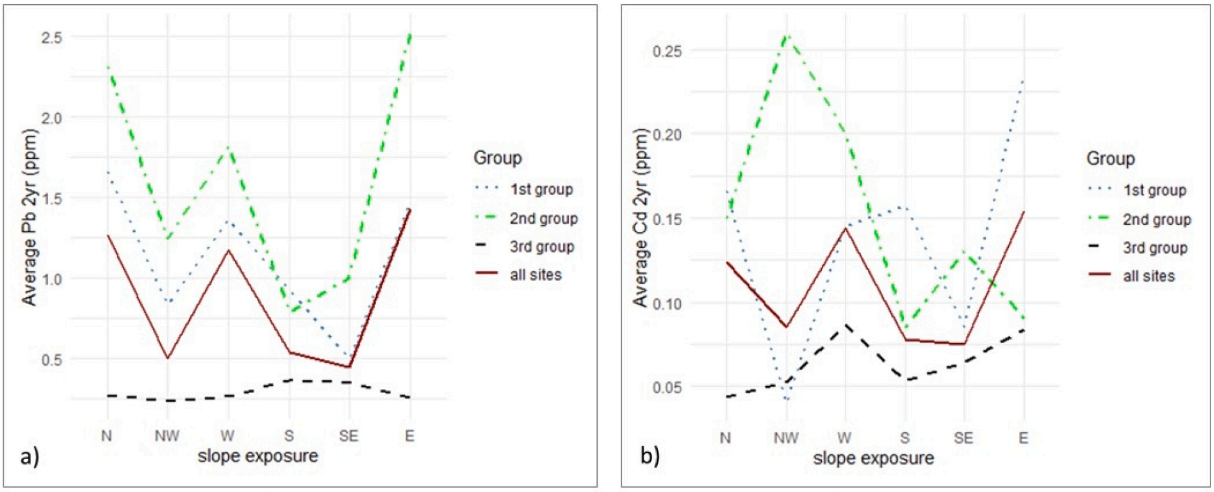

Fig. 9. Average Pb (a) and Cd (b) concentrations (ppm) in two-year-old needles of *Pinus mugo* in 2011 at **all sites** (red solid line); in the **1st group** of sites (blue dotted line): Little Fatra Mts. (Martinske hole Mt., Suchy Mt., Krivan Mt.), Oravske Beskydy Mts. (Babia hora Mt.), Chocske vrchy Mts. (Choc Mt.); in the **2nd group** of sites (green dotted-dashed line): Great Fatra Mts. (Borisov Mt., Krizna Mt.), Low Tatra Mts. (Kralova hola Mt.); in the **3rd group** of sites (black dashed line): Belian, High and Westhern Tatra Mts. (Bujaci vrch Mt., Velke Biele pleso Lake, Zelene pleso Lake, Zadne Medodoly Valley, Velicka dolina Valley, Brestova Mt., Sivy vrch Mt.) - depending on slope exposure. (95% confidence). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

local contamination from the high industrial areas of central Slovakia. The northern slopes were also affected by local pollutants. Kralova hola Mt. had the highest Pb value on this slope (site 56: 2.31 ppm).

Sites in the Tatra Mountains (3rd group) were the most burdened by Pb on the southern and south-eastern slopes. The highest value was measured on southern slope of Brestova Mt. (site 60: 1.03 ppm). In Western Tatras, more lead came from local sources (traffic, industry) compared to the northern slopes affected by transboundary pollutants. The highest Pb concentrations in the Belian and High Tatra Mts. within southern and south-eastern slopes on the Kopske sedlo Saddle (S – 0.40 ppm, SE – 0.69 ppm) were caused by emissions from two directions – transboundary (southern Poland) and local (district of Poprad). The pollutant stream hit the mountain massif and then turned to the southern slope of monitored sites (Fig. 10). Then a gradual decrease of Pb was found from west to east. More details are in the Appendix (Text SI 4).

An increasing Cd gradient can in general be seen southeast from Northern Europe (Pacyna et al., 2009). In our study, Cd (2 yr) had the highest average values on the eastern, western and northern slopes for all analysed sites, 1st group and 3rd group of sites (Fig. 9b). The results of the 1st group showed that Martinske hole Mt. and Suchy Mt. were exposed to highest Cd concentrations on the eastern slopes affected by local pollutants from district of Martin (Turcianska basin). Krivan Mt.

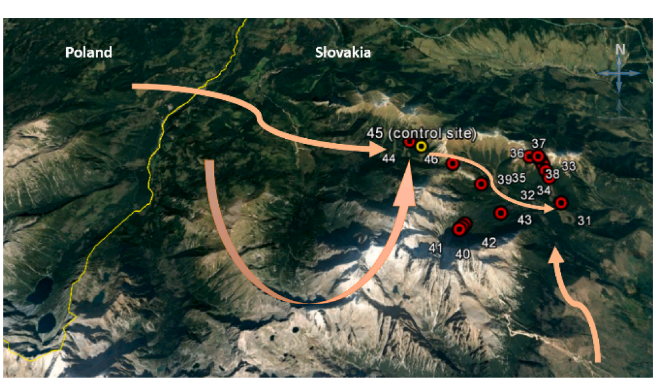

Fig. 10. Direction of Pb and Cd dispersion in the Belian Tatra Mts. and in the easternmost part of the High Tatras (3rd group of sites).

had the highest values on the northern slope. Similarly as Pb, higher Cd concentrations there came from abroad compared to local sources from the south.

Sites in the Great Fatra Mts. and Low Tatra Mts. (2nd group) reached the highest average Cd values on the north-western and western slopes influenced by local industrial pollutants from Turcianska and Hornonitrianska basins (Krizna Mt. and Borisov Mt.) and from industrial cities Ruzomberok and Liptovsky Mikulas (Kralova hola Mt.).

For the 3rd group of mountains, the most highly loaded slopes were western and eastern. The western slope (windward) of Bujaci vrch Mt. in the Belian Tatra Mts. had 4.4 times higher Cd (site 38: 0.22 ppm) than the leeward site (site 37: 0.05 ppm). This clearly showed that the largest source region of Cd was to the west (southern Poland and northern Moravia). Cd pollution distribution in Poland was described by (Dmuchowski and Bytnerowicz, 1995). More details are in the Appendix (Text SI 4).

3.3.3. Heavy metals vs. Altitude

The relationship between heavy metal content (Pb, Cd) accumulated in the pine needles (1 yr, 2 yr old) and increasing altitude is described in Fig. 11. The values measured on this transect were 0.10–0.26 ppm (Pb1yr) and 0.13–0.53 ppm (Pb2yr) for lead and 0.01–0.28 (Cd1yr) and 0.02–0.22 ppm (Cd2yr) for cadmium. The highest Pb and Cd values in both years were measured at the highest sampling point (BVT7; 1,816 m a.s.l) close to the mountain top on the windward (west) side of the slope. A different values of these pollutants were found between the two highest sites (BVT6, 7), which had almost the same altitude but different slope exposures. This confirmed the long–range transboundary transport of the highest concentrations of pollutants from the west (northwest). Bacardit and Camarero (2010) found a boundary 2,300 m a.s.l. in high mountain soils of Central Pyreneas, where only above this altitude could trace elements be considered as background, long-range contamination. There they also measured significantly higher values (Pb, Cd) at the highest altitudes. In our study, the higher values at the lowest sampling point (BVT1) were due to the pollutant dispersion from southern Poland through the Kopske sedlo Saddle (Fig. 10). A positive correlation between heavy metal concentrations and altitude can be observed only in areas with a weak influence of local pollution (Zechmeister, 1995). In our research, a positive correlation between needle Pb (1 yr) content and altitude ($r = 0.955$, $p = 0.045$) was found for sites BVT2 – BVT5. The

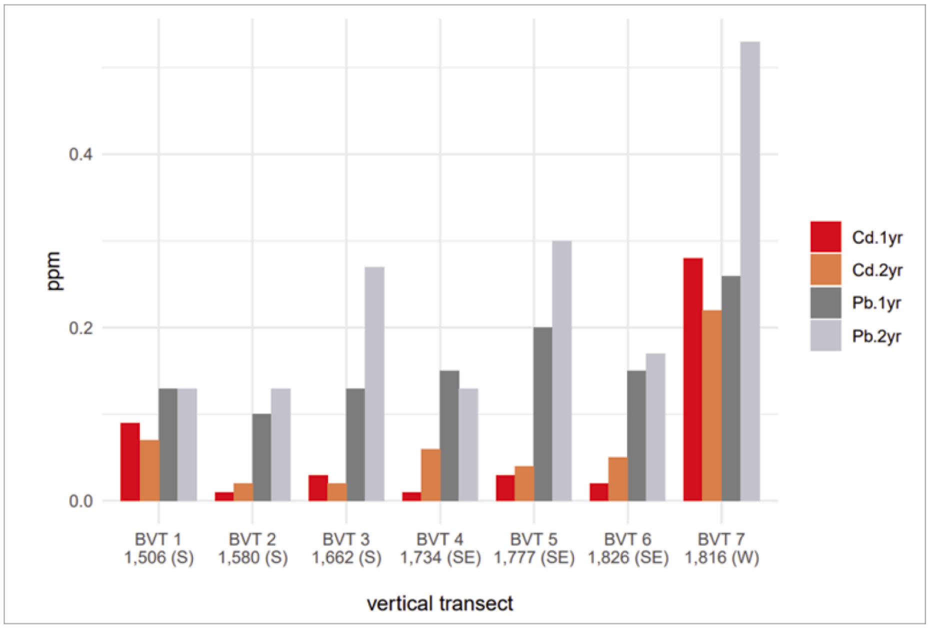

Fig. 11. Lead and cadmium concentrations in one and two-year-old needles of *Pinus mugo* measured along a vertical transect BVT1 - BVT7 (sites 32–38) in Belian Tatra Mts. at altitudes of 1,506–1,826 m a.s.l of Bujaci vrch Mt. with slope exposure, 2011.

lead increase there was observed up to 1,777 m a.s.l. at S-SE exposure. The correlation with altitude has not been confirmed for Pb2 yr, soil Pb and needle Cd content. Bednářová and Bednář (1978) found increased Pb concentration in plants of Tatra Mts. in transects at different distances from the road up to 1,600 m a.s.l. Lee et al. (2005) described a gradual decrease of Pb and Cd levels with increasing altitude up to 1,100 m a.s.l as a result of concentration and transfer of traffic emissions in lower air masses. Chropeňová et al. (2016) evaluated the pine needle Pb on an elevation transect (Western Tatra Mts.) which was more influenced by local industry than in our study. A positive correlation between altitude and heavy metal deposition in the Tatra Mts. was reported by Šoltés (1998). According to Smidt et al. (1996), the increase of heavy metals with altitude (Tyrolean Alps) was related to the increasing amount of precipitation. Wet deposition represents the main pathway of Pb and Cd to plants in the mountains. A significant positive correlation of Pb in *Calluna vulgaris* tissues with increasing altitude was also found on the south slope of Lomnicky stit Mt. (High Tatra Mts.), but Cd there similarly as in our research did not show a correlation (Šoltés et al. 2014).

The distribution of heavy metal atmospheric deposition in mountain habitats depends on altitude, meteorological conditions and orography (Ciriaková, 2009). The slope aspect is an important factor affecting heavy metal deposition. Our results of Pb2yr showed alternation of higher and lower values probably caused by high slope degree. The range between the individual sampling sites 50–70 altitudinal meters was very short for so steep slope. The influence of slope degree as another factor affecting Pb accumulation should be evaluated in further research. In study of Gerdol et al. (2002) the correlation between Cd concentration in moss *Hylocomium splendens* (Southern Alps) and altitude was not found. The Pb showed a decline with increasing altitude related to the increase of net primary production (the amount of exchange sites on the cell wall increased less than total biomass). Cellular (Pb - extracellular) location of an element was important for evaluation of accumulation. There, the precipitation and soil concentration of elements were not crucial. Further research noted the relationship between Pb and Cd concentrations in alpine mosses and cloud cover frequency, without precipitation. The highest values were found at mid-elevation sites (1,400–1,800 m a.s.l) where the frequency of cloud cover was the highest (Gerdol and Bragazza 2006).

A link to a GIS visualization of results through online zooming orthophotomap is available in the appendix (Map SI 1).

4. Conclusions

In conclusion, it can be stated that the evaluated Slovak mountains were particularly exposed to local but also to long-range transboundary air pollutants. The pollen abortion assay allowed us to effectively investigate the genotoxic effect of air pollutants (total mixture of pollutants) in the mountain and alpine habitats. It was sensitive enough to indicate the presence of contaminant brought by air currents from distant or even transboundary sources of pollution. This test is effective, simple and cheap. The highest genotoxicity on the western slopes in the 1st group of mountains was mainly caused by transboundary pollutants and local emissions from urban and industrial stationary sources. Mountains of the Great Fatra Mts. (2nd group) showed the strongest genotoxic effect on the southern slopes, which were influenced by emissions from a highly industrialized area called the Horna Nitra loaded region. In the Tatra Mts. region (3rd group) industrial areas of Southern Poland (Krakow), Northern Moravia (Ostrava) and Silesia regions were the major emission sources. Despite higher values, the pollen abortivity did not exceed the limit of 5% at any of the monitored sites. The highest Pb and Cd concentrations in pine needles were measured in the Great Fatra Mts. on Krizna Mt. surrounded by highly industrialized areas. The relationship between pollen abortivity and Pb content was found only at tops of mountains. The influence of established factors (geology subsoil type and altitude) on pollen abortivity and heavy metal (Pb) values was proved. The lead content increased with needle age. Based on results, it can be stated that the studied Slovak mountains were not significantly burdened by these pollutants. Pollen grains and *Pinus mugo* needles have proven to be suitable bioindicators for air quality assessment in mountain areas. The combination of the environmental genotoxicity evaluation (pollen abortivity) and chemical analyses of plant tissues represents a valuable methodological approach.

CRediT authorship contribution statement

Eva Klemmová Gregušková: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft.

Daniel Mihálik: Formal analysis, Methodology. **Ján Kraic:** Formal analysis, Methodology. **Michaela Mrkvová:** Formal analysis, Methodology. **Jozef Sokol:** Formal analysis, Methodology. **Petr Gregor:** Formal analysis, Methodology, Investigation. **Aneta Rafajová:** Data curation, Methodology. **Pavel Čupr:** Conceptualization, Supervision, Methodology, Validation, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109009.

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