

IN SITU ASSESSMENT OF POLLEN ABORTIVITY IN *ARABIDOPSIS THALIANA* POPULATIONS NEAR INDUSTRIAL COMPLEXES

IN SITU HODNOTENIE PEĽOVEJ ABORTIVITY V POPULÁCIÁCH *ARABIDOPSIS THALIANA* V BLÍZKOSTI PRIEMYSELNÝCH KOMPLEXOV

Karol Mičieta*, Michal Hrabovský

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany,
Révová 39, 81102 Bratislava, Slovakia

Received 10 May 2026; Received in revised form 31 May 2026; Accepted 1 June 2026

Abstract

Long-term monitoring of environmental genotoxicity was conducted using pollen abortivity in wild populations of *Arabidopsis thaliana* occurring in industrially impacted and reference areas in Slovakia. The study focused on a highly contaminated site near an aluminium plant in Žiar nad Hronom and comparative locations along an industrial–urban gradient in Bratislava and the Danube floodplain (Kalinkovo village). Pollen abortivity was evaluated over a 34-year period using standardized cytological methods. Statistical analysis based on linear mixed-effects modelling revealed a significant negative temporal trend in mutation counts ($p < 0.001$), with consistent decreases observed across all monitored localities. The highest genotoxic load was recorded at the aluminium plant site, while reference locations exhibited lower and more stable values. A gradual reduction in pollen abortivity was observed in all areas, likely reflecting improved environmental conditions, including industrial emission reductions and implementation of cleaner production technologies. The results confirm the sensitivity of *A. thaliana* pollen abortivity as a bioindicator of environmental genotoxicity and demonstrate its applicability in long-term field biomonitoring studies.

Key words: *Arabidopsis thaliana*; pollen abortivity; ecogenotoxicity; biomonitoring; industrial pollution; linear mixed-effects model; long-term monitoring; genotoxic stress

Introduction

Currently, ecosystems are exposed to complex and long-term mixtures of xenobiotics originating primarily from anthropogenic activities. The identification and monitoring of environmental contamination are essential prerequisites for its assessment, management, and mitigation. In this context, a wide range of methods for evaluating environmental health has been developed, with particular emphasis on detecting the genotoxic effects of pollutants (Bianchi et al. 2011; Salles et al. 2016; Iqbal et al. 2019).

Higher plants represent important model organisms in biomonitoring because they integrate environmental influences at both the organismal and genetic levels (De Serres

* Corresponding author: karol.micieta@uniba.sk

1978; Grant 1998). Plants also possess metabolic capabilities that may convert certain xenobiotics into more reactive, potentially genotoxic metabolites, which can subsequently enter trophic chains (Lakes, Kim 2012). Their use in in situ environmental monitoring is supported by their sensitivity to genotoxic agents, their ability to metabolize certain contaminants, and their ease of experimental handling (Käffer et al. 2012; Mišík et al. 2019). Plant-based test systems enable the detection of a broad spectrum of environmental stressors, including mutagenic and ecotoxic effects (Mičieta, Murín 1996, 2018; Mičieta 2021). The use of plants as model systems for environmental ecotoxicity and ecogenotoxicity testing has been reported by several authors, including Paradiž, Lovka (1999), Pogányová et al. (2017, 2019), Mičieta, Murín (2018).

One of the widely used model species is *Arabidopsis thaliana* (L.) Heynh., which is extensively applied in studies of induced mutagenesis and ecogenotoxicity due to its well-characterized biology and availability of genetic tools. Important endpoints include the analysis of embryonic and chlorophyll mutations in seeds as well as reproductive parameters such as pollen abortivity (Müller 1963; Müller, Gichner 1964; Gichner et al. 1994; Rédei 1982; Rédei et al. 1984; Gichner, Velemínský 1988). Methodologies based on *A. thaliana* allow standardized assessment of genotoxic effects under both laboratory and field conditions (Sanchez-Serrano, Salinas 2021; Chroust et al. 1997; Abramov; Schevchenko 2005).

In continuation of these approaches, we present results from long-term monitoring of pollen abortivity in native populations of *A. thaliana* occurring in the vicinity of industrial complexes, aiming to evaluate their potential as indicators of environmental pollution.

Methods

Study area

Arabidopsis thaliana does not compete successfully in dense grassland vegetation and typically occurs in disturbed habitats. It preferentially colonizes microsites with bare or disturbed soil, such as erosion patches or mechanically disturbed areas, often within communities of the class *Stellarietea mediae* Tüxen et al. ex von Rochow 1951.

For the assessment of ecogenotoxicity, an exposed site was selected in 1991 in the vicinity of the aluminium plant in Žiar nad Hronom, near the abandoned village of Horné Opatovce (central Slovakia). At the beginning of the monitoring period, the aluminium plant in Žiar nad Hronom represented a major industrial source of pollution, emitting high concentrations of SO₂, PCBs, and polycyclic aromatic hydrocarbons (PAHs). For example, concentrations in plant biomass reached 2.0 mg·kg⁻¹ for phenanthrene, 3.3 mg·kg⁻¹ for fluoranthene, 1.7 mg·kg⁻¹ for pyrene, 1.4 mg·kg⁻¹ for chrysene, and 3.1 mg·kg⁻¹ for perylene (Mičieta et al. 1997). During this period, total emissions exceeded 15,000 tonnes, including approximately 10,000 tonnes of gaseous emissions. Additional releases included 800–1,000 tonnes of fluoride compounds, 42.5 tonnes of arsenic, 800 tonnes of tar and phenolic compounds, 0.86 tonnes of mercury, and other pollutants. In 1995, production was modernized by introducing pre-baked technology, and industrial

activity was completely terminated in 2023.

For comparative purposes, reference sites were selected in the vicinity of an oil refinery and a municipal waste incinerator in Bratislava. Sampling was conducted at three sites, representing a spatial gradient along the Danube floodplain: Bratislava–Biskupické rameno, Kalinkovo–Bajdelský most, and Kalinkovo–Biskupická hrádza (Fig. 1). Current concentrations of air pollutants (e.g. SO₂, NO_x, PM₁₀, benzene) were obtained from the Slovak Hydrometeorological Institute. Although the sites in Kalinkovo exhibited some level of anthropogenic influence, they can be considered a near-reference locations due to relatively low pollutant exposure.

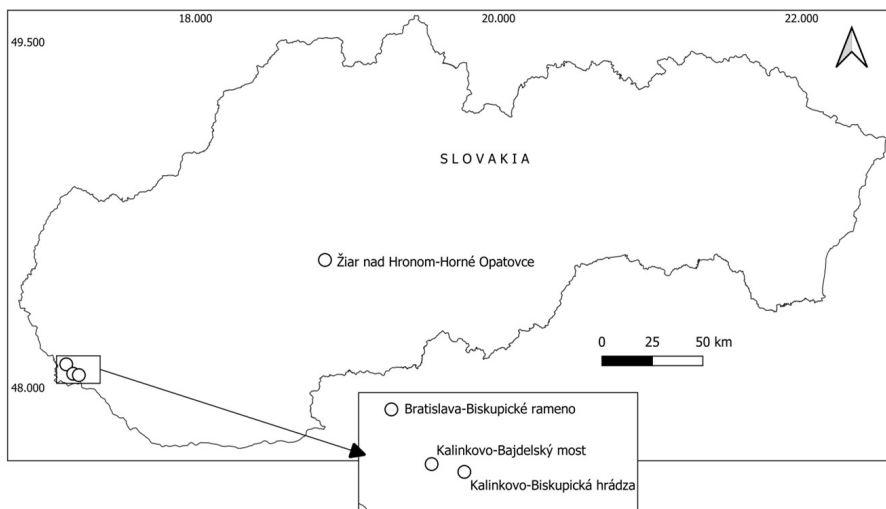


Fig. 1 Study area with sampling sites.

Pollen sampling and preparation

Sampling, slide preparation, and evaluation of pollen abortivity and pollen anomalies were performed according to established protocols (Mičieta, Murín 1996, 1997, 2018). Young closed or partially open flowers were collected and fixed in a solution of 96% ethanol and glacial acetic acid (3:1, v/v). After 10 minutes (maximum 1 h), samples were transferred to 70% ethanol. Pollen grains were carefully released from anthers under a stereomicroscope onto microscope slides. Slides were stained with 1% aniline blue dissolved in lactophenol and mounted with cover slips. For each sample, at least 10 individuals and a minimum of 3,000 pollen grains were evaluated.

Statistical analysis

Mutation counts were analysed using a linear mixed-effects modelling framework to account for both temporal effects and repeated measurements across localities. a linear mixed-effects model was fitted with mutation count (value) as the response variable and year (year) as a fixed effect. Locality (locality) was included as a random intercept to account for between-site variability in baseline mutation levels. To account for heteroscedasticity arising from differing measurement precision, observations were weighted using the inverse of their associated standard deviations, implemented via

a fixed variance structure. To evaluate whether temporal trends differed among localities, an extended model including random slopes for year by locality was also fitted. Model performance was compared between the random-intercept model and the random-slope model using likelihood ratio tests and information criteria (AIC and BIC).

Model assumptions, including normality and homogeneity of residuals, were assessed through diagnostic plots of standardized residuals. All analyses were conducted using the lme4 and nlme packages in R.

Results

Descriptive statistics of mutation counts across localities are summarized in Table 1. Mean values differed among localities, with the highest average mutation count observed in Žiar nad Hronom–Horné Opatovce (mean = 4.69), followed by Bratislava–Biskupické rameno (mean = 3.45), Kalinkovo–Biskupická hrádza (mean = 2.98), and Kalinkovo–Bajdelský most (mean = 2.79). Variability also differed between localities. Žiar nad Hronom–Horné Opatovce exhibited the highest standard deviation (SD = 1.26), indicating greater dispersion of values, whereas Kalinkovo–Bajdelský most and Kalinkovo–Biskupická hrádza showed comparatively low variability (SD = 0.279 and 0.267, respectively). Bratislava–Biskupické rameno showed intermediate variability (SD = 0.753). The range of observed values varied across localities, with Žiar nad Hronom–Horné Opatovce spanning from 2.94 to 6.83, while the other localities showed narrower

Table 1 Mean mutation counts by year with corresponding standard deviations (SD). HO: Žiar nad Hronom–Horné Opatovce, BR: Bratislava–Biskupické rameno, BM: Kalinkovo–Bajdelský most, BH: Kalinkovo–Biskupická hrádza

Year	Locality			
	HO	BR	BM	BH
1991	6.83±1.81	4.42±0.62	3.28±0.32	3.52 ± 0.22
1994	6.43±1.2	4.64±0.80	3.28±0.32	3.26±0.26
1997	6.02±0.42	4.58±0.28	3.05±0.43	2.84±0.32
1999	5.38±0.85	3.36±0.24	2.98±0.19	3.02±0.24
2004	4.90±0.72	3.68±0.32	2.74±0.25	2.54±0.22
2006	5.03±0.62	3.22±0.26	2.84±0.31	3.20±0.42
2008	4.66±0.43	3.62±0.36	2.65±0.21	2.86±0.34
2010	4.40±0.59	3.84±0.26	2.56±0.27	3.02±0.32
2012	4.26±0.41	2.86±0.34	2.62±0.38	2.82±0.43
2015	3.94±0.34	2.88±0.36	2.48±0.44	3.20±0.38
2019	3.18±0.26	2.74±0.40	2.50±0.30	2.66±0.28
2024	2.94±0.32	2.48±0.20	2.56±0.46	2.82±0.22
2025	3.02±0.22	2.58±0.42	2.69±0.22	3.02±0.42

ranges (e.g., Kalinkovo–Bajdelský most: 2.48–3.28; Kalinkovo–Biskupická hrádza: 2.54–3.52). Relative variability, expressed as the coefficient of variation, was highest in Žiar nad Hronom–Horné Opatovce (CV = 0.268), followed by Bratislava–Biskupické rameno (CV = 0.218) and Kalinkovo–Bajdelský most (CV = 0.100), while Kalinkovo–Biskupická hrádza showed the lowest relative variability (CV = 0.089). Overall, Žiar nad Hronom–Horné Opatovce not only exhibited the highest mean mutation count but also the greatest variability, whereas Kalinkovo–Biskupická hrádza and Kalinkovo–Bajdelský most were characterized by lower and more stable mutation levels (Fig. 2).

A linear mixed-effects model was fitted to evaluate the effect of year on mutation counts while accounting for variability among localities. Locality was included as a random intercept, and observations were weighted using their associated standard deviations to address heteroscedasticity. The model revealed a strong and statistically significant negative effect of year on mutation counts ($\beta = -0.04176$, SE = 0.00615, $t = -6.79$, $p < 0.001$), indicating a pronounced decrease in mutation counts over time (Fig. 3). The random effects indicated variability among localities in baseline mutation levels (SD = 0.694), while residual variability was slightly lower (SD = 0.799), suggesting moderate within-group variation relative to between-locality differences.

To assess whether temporal trends differed among localities, a model including random slopes for year was compared to the random-intercept model. The comparison showed no improvement in model fit (likelihood ratio test: $\chi^2 \approx 0$, $p = 1$; AIC = 99.26 for the random-intercept model vs. 103.26 for the random-slope model), indicating that allowing slopes to vary by locality did not better explain the data. This suggests that the temporal decline in mutation counts is consistent across all localities.

Overall, the results demonstrate a strong and significant decreasing trend in mutation counts over time, with differences between localities primarily reflected in baseline levels rather than in their temporal dynamics.

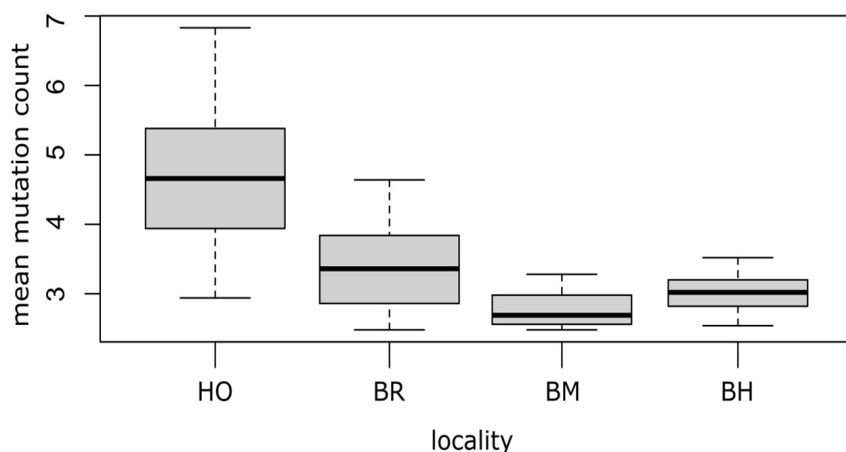


Fig. 2. Mutation counts by locality. Boxplots represent the distribution of values for each locality, with medians, interquartile ranges, and whiskers indicating variability. HO: Žiar nad Hronom–Horné Opatovce, BR: Bratislava–Biskupické rameno, BM: Kalinkovo–Bajdelský most, BH: Kalinkovo–Biskupická hrádza

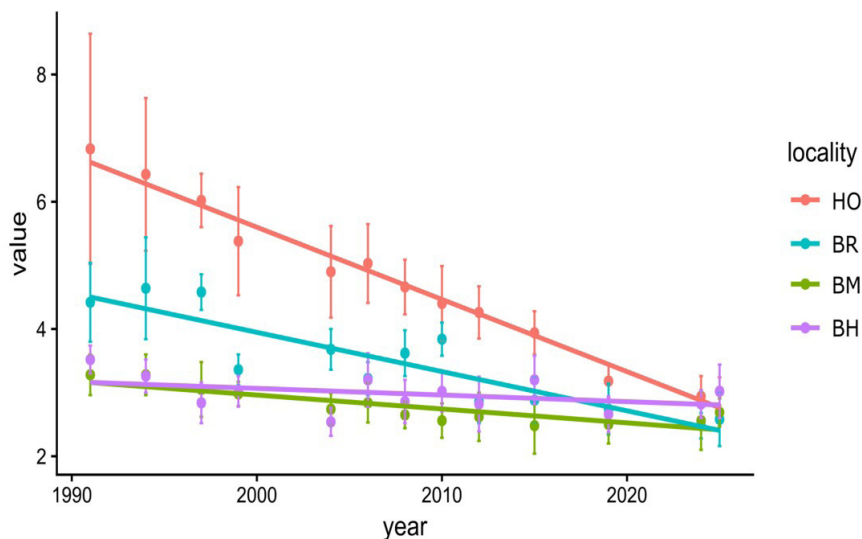


Fig. 3. Temporal variation in mutation counts across localities. Points show observed values, with error bars indicating $\pm$ standard deviation (SD). Solid lines represent fitted linear trends from a linear mixed-effects model including locality as a random intercept. Colours denote different localities. HO: Žiar nad Hronom–Horné Opatovce, BR: Bratislava–Biskupické rameno, BM: Kalinkovo–Bajdelský most, BH: Kalinkovo–Biskupická hrádza

Discussion

The results indicate increased pollen abortivity at the site near the aluminium plant, suggesting a genotoxic effect of industrial emissions on the surrounding environment. Similar findings have been reported in studies using indicator species of wild flora and in long-term tetrad analyses of *Calluna vulgaris* (Mičieta, Murín 1999). a clear decreasing trend in pollen abortivity was observed across all monitoring sites over the 34-year observation period. This trend can be partly explained by the cessation of industrial activity at the aluminium plant in 2023. a similar, although less pronounced, trend was observed in the vicinity of the oil refinery and municipal waste incinerator (Bratislava and Kalinkovo), where exposure to industrial–urban emissions was associated with a gradual decline in pollen abortivity and reduced ecogenotoxic stress, likely reflecting the effects of environmental mitigation measures implemented at the incineration facility.

For comparison, *Arabidopsis thaliana* plants cultivated ex situ at the Botanical Department, Bratislava (Révová Street), were grown from seeds collected in the impacted area. These plants showed relatively stable mean pollen abortivity values over time: 3.02% (1991), 2.99% (2006), 2.6% (2010), and 2.14% (2023) (unpublished data), suggesting a gradual decline in genotoxic stress in the studied environments. This is consistent with results obtained from indicator species of wild flora and from the model species *Tradescantia paludosa* E. S. Anderson et Woodson (Mišík et al. 2007).

According to standardized protocols for mutagenicity assessment using wild plant

indicators (Mičieta, Murín 1996), pollen abortivity values below 5% are considered acceptable. Long-term monitoring of pollen abortivity in wild populations of *A. thaliana* confirms the suitability of this species as an indicator of ecogenotoxicity in polluted environments, particularly when complemented by assays detecting chlorophyll and embryonic mutations. It also represents a useful additional bioindicator alongside standardized models such as *Tradescantia paludosa* and clone 4430. However, the use of wild *A. thaliana* populations has certain limitations. Habitat disturbance and vegetation succession affect population stability, leading to irregular or reduced occurrence at sampling sites. This is particularly evident at the Kalinkovo–Biskupická hrádza site of the Gabčíkovo Waterworks, where increasing vegetation cover has led to a decline in this species.

Conclusion

Long-term monitoring of pollen abortivity in wild populations of *Arabidopsis thaliana* demonstrated clear spatial and temporal patterns of environmental genotoxicity across industrially influenced and reference sites. The highest levels of pollen abortivity were recorded at the site affected by historical emissions from the aluminium plant in Žiar nad Hronom, confirming its role as a strong anthropogenic stressor in the past. a statistically significant decreasing trend in mutation and abortivity levels was observed over the 34-year study period across all monitored locations. This trend is consistent with improvements in industrial technologies, reduction of emissions, and implementation of environmental protection measures, particularly at major industrial sources in the region.

Comparative analyses further confirmed similar but weaker patterns in areas influenced by refinery and municipal incineration emissions.

Overall, the results support the applicability of plant-based biomonitoring systems for long-term assessment of environmental genotoxicity and demonstrate a measurable improvement in environmental quality across the studied regions.

References

- Abramov, V. I., Rubanovich, A. V., Shevchenko, V. A., 2005: Genetic Effects of Low-Dose Chronic Irradiation of Developing Seeds of *Arabidopsis thaliana* (L.) Heynh. Russian Journal of Genetics, 41(9): 1021–1027.
- Bianchi, J., Espindola, E. L. G., Marin–Morales, M. A., 2011: Genotoxicity and mutagenicity of water samples from the Monjolinho River (Brazil) after receiving untreated effluents. Ecotoxicology and Environmental Safety, 74: 826–833.
- Chroust, K., Kuglík, P., Relichová, J., Holoubek, I., Čáslavský, J., Veselská, R., Rysková, M., Benedík, J., 1997: *Drosophila melanogaster*, *Vicia faba* and *Arabidopsis thaliana* short-term bioassays in genotoxicity evaluation of air and soil samples from sites surrounding two industrial factories in the Czech Republic. Folia Biologica (Praha), 43: 71–78.
- De Serres, F. J., 1978: Utilization of higher plant systems as monitors of environmental mutagens. Environmental Health Perspectives, 27:3–6.
- Gichner, T., Badayev, S. A., Demchenko, S. I., Relichova, J., Sandhu, S. S., Usmanov, P. D., Usmanova O., Velememinský, J., 1994: *Arabidopsis* assay for mutagenicity. Mutation Research, 310: 249–256.
- Gichner, T., Veleminský, J., 1988: Testovací systém na embryonální a chlorofýlové mutace: *Arabidopsis thaliana*. Acta Hygienica, Epidemiologica et Microbiologica, 16: 133–136.

- Grant, W. F.**, 1998: Higher plant assays for the detection of genotoxicity in air polluted environment. *Ecosystem Health*, 4: 210–229.
- Iqbal, M., Abbas, M., Nisar, J., Nazir, A.**, 2019: Bioassays based on higher plants as excellent dosimeters for ecotoxicity monitoring: a review. *Chemistry International*, 5(1): 1–80.
- Käffer, M. I., Lemos, A.T., Apel, M. A., Rocha, J. V., de Azvedo Martins, S. M., Ferrão Vargas, V. M.**, 2012: Use of bioindicators to evaluate air quality and genotoxic compounds in an urban environment in Southern Brazil. *Environmental Pollution*, 163: 24–31.
- Lakes, T., Kim, H-O.**, 2012: The urban environmental indicator “Biotope Area Ratio”—An enhanced approach to assess and manage the urban ecosystem services using high resolution remote-sensing. *Ecological Indicators*, 13: 93–103.
- Mičieta, K.**, 2021: Phytoindication of environmental mutagenesis in the in-situ conditions using the pollen of selected species of native flora. *Thaiszia*, 31(1): 69–78.
- Mičieta, K., Murín, G.**, 1996: Microspore analysis for genotoxicity of a polluted environment. *Environmental and Experimental Botany*, 36: 21–27.
- Mičieta, K., Murín, G.**, 1997: Wild plant species in practical use for bioindication of polluted environment. *Ekológia (Bratislava)*, 16: 193–202.
- Mičieta, K., Murín, G.**, 1999: Retrospective biomonitoring: a hundred years of environmental pollution at selected areas in Slovakia. *Journal of environmental pathology, toxicology and oncology*, 18: 335–338.
- Mičieta, K., Murín, G.**, 2018: In situ biomonitoring of a polluted environment by wild plant and crop plant species. Nova Science Publishers, Inc., New York, 224 pp.
- Mičieta, K., Ostrovský, I., Murín, G.**, 1997: Organic Xenobiotics and Plants: Impact, Metabolism and Toxicology. In: Weiss, P., Schröder, P., Rether, B., Keith, G., Collins, C. Bach, Th. (eds.), *Proc. of the IMTOX'97 (Vienna)*, 24, pp. 97–104.
- Mišík, M., Isidori, M., Umbuzeiro, G.**, 2019: Ecotoxicology: Conventional and new topics and methods. *Mutation Research*, 842: 1–2.
- Mišík, M., Mičieta, K., Solenská, M., Mišíková, K., Pisarčíková, H., Knasmüller, S.**, 2007: In situ biomonitoring of the genotoxic effects of mixed industrial emissions using the *Tradescantia* micronucleus and pollen abortion tests with life plants. Demonstration of the efficacy of emission controls in an eastern European city. *Environmental Pollution*, 145: 459–466.
- Müller, A. J.**, 1963. Embryonentest zum Nachweis rezessiver Letalfaktoren bei *Arabidopsis thaliana*. *Biologisches Zentralblatt*, 82: 113–182.
- Müller, A. J., Gichner, T.**, 1964: Mutagenic Activity of 1–Methyl–3–nitro–1–nitrosoguanidine on *Arabidopsis*. *Nature*, 201: 1149–1150.
- Paradiž, J., Lovka, M.**, 1999: Pollen grain bioassay for environmental pollution screening. *Phyton, Annales Rei Botanicae*, 39: 175–182.
- Pogányová, A., Kerekeš, E., Mičieta, K.**, 2017: The ecogenotoxic plant biomonitoring of a long-term polluted area in central Slovakia. *Environmental Science and Pollution Research*, 24: 27376–27383.
- Pogányová, A., Mičieta, K., Dušička, J.**, 2019: Genotoxic assessment of selected native plants to differentially exposed urban ecosystems. *Environmental Science and Pollution Research*, 26: 9055–9064.
- Rédei, G. P.**, 1982: Mutagen assay with *Arabidopsis*. a report of the U.S. Environmental Protection Agency. Gene-Tox Program. *Mutation Research*, 99: 243–255.
- Rédei, G. P., Acedo, G. N., Sandhu, S. S.**, 1984: Mutation induction and detection in *Arabidopsis*. In: Chu, E. H. Y., Generoso, W. M. (Eds). *Mutation, cancer, and malformation*. New York, Plenum, pp. 285–313.
- Salles, F. J., de Toledo, M. C. B., César, A. C. G., Ferreira, G. M., Barbério, A.**, 2016: Cytotoxic and genotoxic assessment of surface water from São Paulo State, Brazil, during the rainy and dry seasons. *Ecotoxicology*, 25: 633–645.
- Sanchez-Serrano, J. J., Salinas, J.**, 2021: *Arabidopsis* Protocols. 4th rev. ed. *Methods in Molecular Biology* 2200, Springer protocols. Humana Press Inc., 465 pp.

Abstrakt

Dlhodobé monitorovanie environmentálnej genotoxicity bolo realizované na základe hodnotenia abortivity peľu v prirodzených populáciách *Arabidopsis thaliana* v priemyselne ovplyvnených a referenčných oblastiach Slovenska. Štúdia sa zamerala na vysoko kontaminovanú lokalitu v okolí hlinikárne v Žiari nad Hronom a porovnávacie lokality pozdĺž industriálno-urbánneho gradientu v Bratislave a na Podunajskej nížine (oblasť Kalinkova). Abortivita peľu bola hodnotená počas 34-ročného obdobia pomocou štandardizovaných cytologických metód. Štatistická analýza založená na lineárnych zmiešaných modeloch preukázala signifikantný negatívny časový trend v počte mutačných prejavov ($p < 0,001$), pričom pokles bol konzistentne pozorovaný na všetkých sledovaných lokalitách. Najvyššia genotoxická záťaž bola zaznamenaná v oblasti hlinikárne, zatiaľ čo referenčné lokality vykazovali nižšie a stabilnejšie hodnoty. Vo všetkých oblastiach bol pozorovaný postupný pokles abortivity peľu, pravdepodobne v dôsledku zlepšenia environmentálnych podmienok, vrátane zníženia emisií z priemyselných zdrojov a zavedenia ekologickejších výrobných technológií. Výsledky potvrdzujú citlivosť abortivity peľu *A. thaliana* ako bioindikátora environmentálnej genotoxicity a demonštrujú jeho využiteľnosť v dlhodobých terénnych biomonitorovacích štúdiách.

Kľúčové slová: *Arabidopsis thaliana*; abortivita peľu; ekogenotoxicita; biomonitoring; priemyselné znečistenie; lineárne zmiešané modely; dlhodobé monitorovanie; genotoxická záťaž